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A COMPARATIVE STUDY OF THE CHROMOSOMES OF MAMMALS[1]

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INTRODUCTION

DURING a series of studies upon mammalian spermatogenesis the writer has made careful counts of the chromosome number in at least one species in seven out of the nine eutherian orders. The primary object of these investigations was to discover whether or not sex is determined in man and other mammals, as in the invertebrates, by definite sex chromosomes. An accurate knowledge of both the form and the number of the diploid chromosomes was, of course, a very important link in the chain of evidence needed to prove the presence of such sex determiners in any animal. Once it was firmly established, however, that sex chromosomes occur in two such extremes as the marsupials on the one hand and the primates on the other, this question of sex determination no longer entirely dominates our interest, and other phases of mammalian cytology appear in the foreground as progress is made in the study of the chromosomes of this group.

At the present time mammalian chromosome studies, aside from the question of sex determination, have both a

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practical and a theoretical interest and value to biologists. From the practical side the exact determination of both the form (especially size relations) and the number of chromosomes of domestical animals gives the cytological foundation upon which the geneticists and practical breeders of the future must build. Of the many theoretical aspects, perhaps the one of dominant interest centers about the behavior which the chromosomes have shown during the evolution of the several mammalian orders. Is there any evidence to show that very marked and extensive changes in the chromosomes have occurred which might have played a rôle in the evolution of these smaller groups? This general question is receiving a great deal of attention from students of plants, and many botanists of to-day are seeking to explain the formation of new species among plants by relatively gross changes in the chromosome complex. Classic examples of this are *Oenothera gigas,* which is a tetraploid race of *Oe. lamarckiana* and *Oe. lata,* which has one more chromosome than *Oe. lamarckiana* and is thought to have been formed from the latter by the addition of one chromosome which presumably arose in meiosis through the nondisjunction of one of these elements. Equally as striking are the observations of Blakeslee and Belling in the Daturas. Numerous other cases are cited and discussed in two recent papers by Gates (24a, 24b) and even at this early time there seems to be no question but that polyploidy and the duplication of one or more chromosomes have played rôles, perhaps very important ones, in the formation of new species among plants.

The eutherian mammals are such a well-unified group that few zoologists doubt that they have arisen from a common stem. This means, of course, that this common stem must have carried the same complement of chromosomes and of genes within the chromosomes. As this ancestral stock diverged into the nine orders we know to-day, how have the primitive chromosomes behaved? Will we find that certain or all of the chromomere associations have been very stable, so that we will be able to

recognize the same chromosome in different orders? Or, on the other hand, have some of the chromosomes been lost, or broken up, or fused, or perhaps undergone both of the latter processes with the formation of entirely new associations of chromomeres and of genes? Have either polyploidy or the duplication of one or more chromosome pairs played a rôle here? At the outset it would be impossible to predict an answer to any of these questions or whether or not extended researches would give us even a hint with regard to them.

There will doubtless be some who will regard this evolutionary aspect of the chromosomes as extremely problematical since, especially in the insects, there is so little apparent order in the great maze of cytological data which has accumulated over a period of thirty years. But the insects, in addition to being extremely old phylogenetically, have undergone a degree of specialization which we do not find in the mammals. Many of the insect orders were fully established long before the mammals appeared on the earth, and while in our classification we regard the mammals, in a general way, as roughly equivalent to the insects, they are really more nearly comparable to an insect order. And within these smaller groups of insects, there is a basic similarity of the chromosomes, as the works of McClung and his students have shown in case of the Orthoptera and Metz and others in the case of Diptera. The extreme youth of the mammals, as compared to some of the insect orders, would lead us to expect that the mammals would prove favorable for determining the general trend in the behavior of the chromosomes during the evolution of this class, if going hand in hand with differentiation into the different orders there has been any uniformity in chromosome behavior.

During the past year the writer has been able to make accurate chromosome counts in three different eutherian orders (insectivors, rodents and edentates) and we now know the chromosome constitution of at least one species in all the orders except the Sirenia and Cetacea. It has

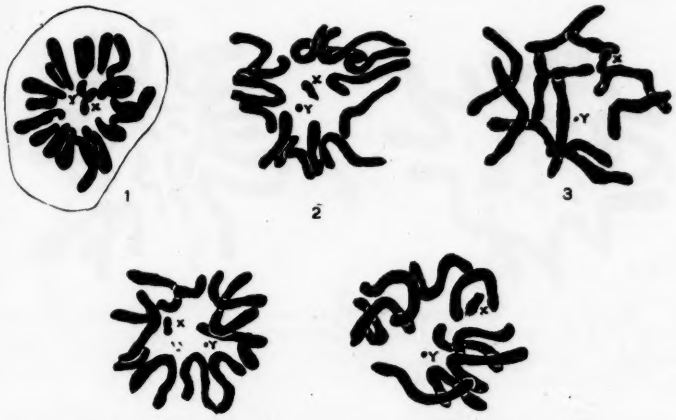
seemed wise to bring together in one place and to discuss from the standpoint outlined above the results which have been obtained during the past five years. I fully realize that many of the questions before us can not be answered with finality, but a discussion of the facts will enable us to see the present status of mammalian chromosome work, and will indicate, perhaps, the lines of study which it will be most profitable to follow in order to gain more light on the general problem of chromosome behavior during evolution.

MARSUPIALS

The marsupials have proved to be the most favorable mammals for chromosome studies, and published works include detailed descriptions of six species from four different families. The number of chromosomes, as compared to the eutheria, is invariably a low one, ranging from 22 in the opossum (Didelphys) to 12 in the kangaroo (Macropus). In Figs. 1 to 5 the spermatogonial chromosomes of five different species are shown. It is to be noted especially that the magnification at which the figures are reproduced is not the same in all cases. I have purposely enlarged the drawings of Agar and of Greenwood, as noted in the legend of the figures, until the size of the X chromosome was about the same for all the species shown.

In the opossum (Fig. 1) there are 22 chromosomes, 20 of which typically form a ring surrounding the small X and Y sex chromosomes. This is the highest number so far encountered in the marsupials.[2] In Phascolarctus (Fig. 2) there are 16 elements, and here again, the large autosomes form a ring about the sex elements. Sarcophilus (Fig. 3) and Dasyurus (Fig. 4) both have 14 chromosomes. In Macropus (Fig. 5) there are 12 elements; this last being the smallest number so far recorded for any mammal.

[2] Agar (*l.c.*) found 22 chromosomes in Petauroides, but since no spermatogonial cells were given in this work, we have been unable to compare this species with the opossum.



Figs. 1 to 5. The spermatogonial chromosomes of five different marsupials are represented, as follows: 1. opossum, 2. Phascolaretus, 3. Sarcophilus, 4. Dasyurus, and 5. Macropus. Figs. 2 to 4 were copied from Greenwood's ('23) Figs. 2, 23 and 27 respectively, and Fig. 5 was copied from Agar ('23) Fig. 3. It is to be noted that these drawings do not represent the same magnification in all cases.

An examination of the figures will show that there is a marked similarity in the chromosome constitution of the marsupials. This is most evident in the case of the sex chromosomes, which are not only of the same type (X–Y) and of the same behavior during meiosis, but are similar in the bilobed condition of the X chromosome. The Y chromosome, as compared to the X element, shows marked differences in size in the different species, being largest in the case of the opossum (Fig. 1) and extremely minute in Sarcophilus, Dasyurus and Macropus. In every case the sex elements are sharply marked off from the autosomes by their small size.

The autosomes, in turn, are alike in general form, all of them being elongated elements much larger than the sex chromosomes. An exact quantitative comparison has not yet been made between either the autosomes or the sex chromosomes of the different species, but if we may use the size of the X chromosome tentatively as a rough measure of chromosome volume, it would seem

Figure 6 is a spermatogonial metaphase plate of Anolis carolinensis. Fig. 7 represents the diploid chromosome of Rana temporaria (copied from Witschi ('24) figure 3, plate XIX).

from the figures that in spite of differences in autosomal numbers in different species, the total bulk of the chromatin in marsupials is not very different. For example, let us consider the two extremes in numbers found in the opossum and in the kangaroo. Using the X chromosome as our standard of comparison, the 10 autosomes of Macropus (Fig. 5) are all much longer than the largest chromosome in the opossum, so that in spite of its low autosomal number Macropus probably carries in its 10 chromosomes a rough equivalent of the opossum autosomal complex.

Further discussion of marsupial chromosomes will be taken up later in this paper.

EUTHERIA

The literature dealing with eutherian chromosomes is filled with discrepancies concerning the chromosome number of most of the species studied. For example, in the case of man the reported number ranges from 16 to 48. It is now clearly recognized by cytologists that earlier workers were handicapped by an inadequate technique to handle mammalian chromosomes, and it would subserve no useful purpose to take up here the literature in detail. Since the development of an adequate technique little work on the eutherian chromosomes has been done except by the writer, so that in the following pages I shall confine

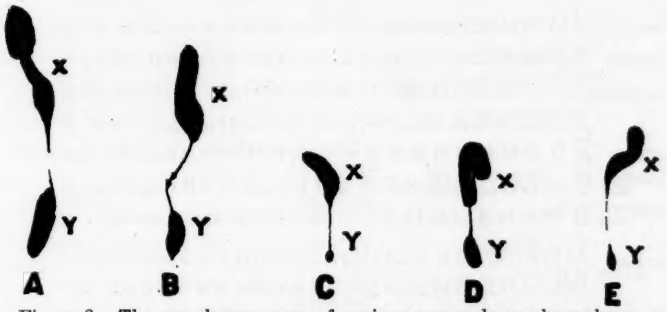


Figure 8. The sex chromosomes of various mammals are here shown as they appear in the first maturation spindle. The magnification is the same in all cases. The forms involved are as follows: A. opossum, B. negro, C. Macacus, D. Cebus, and E. horse.

my descriptions (as regards the eutheria) to those species in which I have made detailed observations. In Study I (Painter '22) a full review of the work prior to 1920 has been given.

In order to facilitate a ready comparison between the chromosomes of different mammals I have placed all the chromosomes of a cell in a horizontal line. This was done in the following way. A drawing is made of the chromosomes of a cell in the ordinary way with the aid of a camera lucida. Then the individual chromosomes of the cell are copied from the drawing on separate bits of cardboard, a copying camera lucida being used for this purpose. In this way one can place chromosomes of similar size and shape together, and beginning with the largest chromosome pair, line all the elements up in a row, approximately in the order of their size. All the chromosomes of Plate I have been drawn and reproduced at the same magnification, which is about 3,600 diameters. Two points must be stressed with regard to the figures on Plate I. In the first place, it must be realized that the pairing up of homologous elements is only approximately correct, especially with medium-sized elements, because in a given cell not all the elements will be in the same plane and one which is tipped on end will appear shorter than it actually is. I have tried to offset this source of

PLATE I

The magnification of all figures of this plate is the same.

error, introduced by foreshortening, by comparing chromosomes under the microscope which appear alike in my drawings. In spite of this unavoidable source of error, however, the majority of chromosomes in a given cell match up in a very striking way, as may be seen for any species represented on Fig. 1. The second point is this, that while all the chromosomes on Fig. 1 are reproduced at the same magnification, the original cells from which they were taken were often of very different sizes. Thus an amnion cell is typically much larger than a germ cell, and chromosomes taken from the former will appear much larger. On Fig. 1, the chromosomes of the bat, armadillo and rabbit were taken from dividing amnion cells, while the other figures were taken from germ cells. Again, the appearance of a given chromosome in different cells of the same individual even is affected by the rate of penetration of the preserving fluid and by slight differences in staining. If the penetration of the preservative is slightly retarded by overlying tissue, the chromosomes appear bulkier and shorter than where more rapid fixa-

tion occurs. Because of these unavoidable differences in technique and of cell size, we can not compare directly the chromosomes in different cells of the same animal or in different species. Our comparisons must be relative, as, for example, the largest pair of chromosomes, or the smallest pair, in different cells.

Primates

My studies in the primates include a description of the spermatogenesis of man, both white and negro (Study II), and of a new and an old world monkey (Study IV). The diploid numbers encountered in these several forms are as follows:

Homo sapiens, white	48
Negro	48
Macacus rhesus	48
Brown Cebus	54

Homo sapiens: As far as I have been able to determine there is no difference between the chromosomes of the white man (Fig. 9) and the Negro (Fig. 10). The chromosome number is the same in both cases and a comparison of the different chromosome pairs, when these are placed in alignment approximately according to their size, shows the same gradual seriation from the largest to the smallest elements. The Y sex chromosome is the smallest body in the cell in both races, and, as I shall show below, the X chromosome is also alike in form and behavior.

Macacus rhesus: The chromosome number of this species is the same as that of man, namely, 48. The chromosomes from two spermatogonial cells are illustrated in Figs. 11 and 12. Due to exceptional preservation the chromosomes of this monkey appear more elongated than in man, but in spite of this one may see that there is the same general seriation in size in the chromosomes of these two primates. The sex chromosomes of the rhesus monkey are of the same type as found in man, the Y being again the smallest elements in the cell. The X chromo-

some of rhesus, as identified in Figs. 11 and 12, appears larger than the X of man. However, in maturation, as I shall show below, when the sex elements can be identified with certainty the X element in man and in the rhesus monkey is about the same size. In spermatogonia it is impossible to identify the X since it has no distinguishing characteristics, and it appears probable that either in man or in the rhesus the true X element is not represented.[3]

Brown Cebus: There are 54 chromosomes in this new world monkey (Fig. 13) including X and Y sex chromosomes. When this chromosome complex is placed in serial alignment, it is evident that as compared to man or the old world form, in cebus the small chromosomes are more numerous and there are correspondingly fewer large elements.

Sex chromosomes of primates: The figures on Plate I indicate that the sex chromosomes of the primates are of the same X–Y type. In the first maturation division, when the sex elements can be identified with certainty, it is evident that they are alike in general form and relative size and show the same behavior. In Fig. 8, B to D, these sex elements are shown as they appear in the first maturation division in man, the rhesus and the cebus monkeys, respectively.

CHEIROPTERA

The species studied was *Nyctinomous mexicanus,* the common house bat of the Austin region. The tissue used for chromosome counts was the amnion of young embryos. There are 48 chromosomes in this species (Fig. 14). When these are paired and lined up in approximately the order of their size and shape, we have a chromosome alignment very similar to that observed in man and the rhesus monkey. It will be noted that the relative size of the bat chromosomes is due to the large size of the amnion cell from which the figure was taken.

[3] As I have pointed out above, matching up of homologous chromosomes can be approximate only. It is clear that in both man and the rhesus monkey there is a medium-sized element which has no mate of like size or shape, but it is impossible to identify the true X at this time in either form.

I have been unable to identify, as yet, the sex chromosomes of the bat in maturation, but the constancy with which 48 chromosomes is found in different embryos, taken together with the fact that in some embryos a pair of very unequal sized elements is observed, would seem to indicate that we have in this form the X–Y type of sex chromosome. The embryo from which Fig. 14 was taken is presumably a male.

Insectivora

The species studied in this order was the common European hedgehog. The testis was the tissue studied and has been reported upon in a short paper (Painter, '25). There are 48 chromosomes in the hedgehog, as Fig. 15 will show. When these are paired up and arranged according to size, we find the same sort of serial alignment observed in the bat and in some of the primates. In the hedgehog the two smallest pairs of chromosomes are more diminutive than in other orders with 48 chromosomes. The sex chromosomes of the hedgehog have not been identified as yet, because of a lack of proper material.

Edentates

The form studied in this order is the armadillo. In an earlier paper (Study II) I reported that, judging from spermatogonial counts, the number of chromosomes in this species was over 50. More recently counts have been made on dividing cells in the amnion. The number of chromosomes present in this form proves to be 60 (Figs. 16 and 17). An examination of the figures will show that there are proportionally more small chromosomes in this animal than in forms with only 48 chromosomes. I have not, as yet, identified the sex chromosomes of the armadillo, since the high number involved, together with the presence of so many small chromosomes, makes the exact study of meiosis extremely difficult.

Ungulata

The form studied in this order was the horse (Study V) in which I found 60 chromosomes. When these ele-

ments are lined up in the approximate order of their size and shape (Fig. 18), it is evident that the small chromosomes are proportionally more numerous than in animals with 48 chromosomes. The sex chromosomes of the horse, as shown by maturation stages (Fig. 8 E), are of the X–Y type, quite similar in general form and behavior to such sex elements in marsupials and primates.

RODENTA

The amnion cells of rabbit embryos were studied in order to determine the chromosome number. Eight different embryos showed consistently 44 chromosomes. Figs. 19 and 20 give the chromosome alignment of two embryos. An inspection of these figures will show that in contrast to such forms as the horse or armadillo, or man even, the rabbit does not have many small chromosomes. The sex chromosomes have not yet been certainly identified in maturation, but embryos all carry the same number of chromosomes (44), and when these are paired up, we find that in some embryos a pair of unequal size remains (Fig. 19), while in others all elements have mates of the same size and shape (Fig. 20). The embryos with the pair of unequal sized elements (Fig. 19) have been interpreted as males with X–Y sex chromosomes.

CARNIVORA

I have studied, in a preliminary way, the testis of the dog. In spermatogonia I am able to count at least 50 chromosomes. Such counts can only be taken as close approximations, but it seems likely that the diploid number in the dog is close to 52.

DISCUSSION

The facts recorded above are of great interest because of the light which they throw on the general question of the behavior of the chromosomes during the evolution of a small group such as the mammals. If the eutheria have come from a common stem which had at one time the same chromosome constitution and genetic complex, as I have

assumed in this article, then a number of questions arise concerning the fate of these primitive elements during the evolution of the various orders. These will be enumerated and discussed below. Back of these questions, however, the matter of central interest is whether or not we can find in the behavior of the chromosomes any evidence for the cause of variations. To express the matter somewhat differently we may ask, Have the changes which have occurred in mammals been due entirely to mutations within the genes, or have extensive and gross changes in the chromatic material played a rôle, such as the botanists are apparently finding in the case of many plants?

As my studies on sex determination have led me to study the chromosomes in different eutherian orders it has become increasingly clear that there is a marked similarity in the chromosome complexes of all the species so far examined. The lowest number which I have observed so far is 44 and the highest 60, but in all these cases there have been the same general size limits, and often a great similarity in the configuration of many of the individual chromosomes. In Plate I, I have brought together and arranged in horizontal rows typical chromosome complexes from species of six different orders, but before entering into a discussion of the facts brought out by this plate, let me again caution the reader that before we can compare the chromosomes from different animals we must take into account differences which are due to cell size and to technique.

The chromosome numbers which I have found in different eutheria are as follows:

Primates	*Homo sapiens* white	48
	Homo sapiens Negro	48
	Macacus rhesus	48
	Brown cebus	54
Cheiroptera	House bat	48
Insectivora	Hedgehog	48
Ungulata	Horse	60
Edentata	Armadillo	60
Rodentia	Rabbit	44
Carnivora	Dog	50 +

The average of these numbers is a fraction over fifty, but the number which I have most frequently found is 48. This is present in the most primitive eutherian order of insectivora, and in such divergent orders as the primates and cheiroptera. The occurrence of 48 chromosomes in three different orders has led me to believe and to tentatively assume that this represents approximately the primitive eutherian number.

When we compare now the chromosome complexes of different species which possess 48 elements, either within an order such as the primates (Figs. 9 to 12) or in different orders (Figs. 9 to 12, 14 and 15) it is apparent that the general seriation of the chromosomes is very similar. Beginning with the largest pair of chromosomes, we have in all cases the same gradual tapering off in sizes. The fact is that in spermatogonial metaphase plates the chromosomes of man, the rhesus monkey and the bat are so similar that one would have difficulty in distinguishing between these species. The same would be true of the European hedgehog, were it not for the diminutive size of the four smallest chromosomes.

The question now arises, Are the 24 pairs of chromosomes found in the primates, cheiroptera and insectivora basically homologous. That is to say, are the different pairs of chromosomes of the same relative size alike in that they carry essentially the same genetic elements? A final answer to this question can only be given by the geneticists of the future. When we are able to plot the chromosome maps of the eutheria as those of the diptera are plotted to-day, then we will have the evidence needed to show that the largest chromosome pair, for example, carries the same genes (in the main at least) in different orders.

In the absence of any genetic evidence on this subject as yet, all the cytologist may do is to point out the inherent probability that these 24 chromosome pairs are alike. This does not mean that very extensive changes have not taken place within the genes themselves, but rather that the skeleton framework of the chromosomes, which is the ma-

terial substratum for the genes, has undergone little morphological change in the species concerned. This conclusion rests upon two general considerations. In the first place, if 48 is about the primitive eutherian number as I have inferred (both because it occurs in three widely different orders and is near the mean of the counts made for species in seven orders) then those animals which show this number have probably retained the primitive complex. It is possible of course that individual chromosomes have undergone changes due to translocation of parts, or even by the loss of entire chromosomes which has subsequently been masked by the fragmentation of other elements, but there is no evidence to indicate that either of these processes has taken place. The chromosomes of different forms with 48 elements are as near alike as we should expect them to be when we consider the limitations in our method of comparison. The second line of evidence is that afforded by the sex chromosomes. In all the cases in which I have been able to identify the sex elements in meiosis they have been alike in type, general form and behavior. From a purely cytological standpoint the close morphological similarity of the sex chromosomes, especially the X element in different mammals, would indicate that this element has undergone little visible change. From a theoretical point of view we might expect this to be the case because, were the X chromosome to undergo a great loss of material, it would probably upset the balance between the autosomes and the sex chromatin and would result in sexually abnormal forms which would be incapable of handing on their peculiar chromosome condition to later generations.

How are we to explain the high chromosome numbers found in the ungulates or the edentates? Two possible explanations are open to us. Either there has been a fragmentation of some of the larger chromosomes, or there has been a duplication of certain chromosome pairs through non-disjunction. The first of these seems the more probable because when we compare the chromosomes of the horse, armadillo or the cebus monkey with

those of man, for example, it is apparent that when there is a larger number than 48, there is an increase in the relative number of small chromosomes, and a corresponding reduction in the number of larger elements. This evidence would seem to amply justify the conclusion that the high chromosome number of the horse or armadillo has been produced by a fragmentation of larger elements which has resulted both in the increase in the total number and in the number of smaller elements. The same explanation would apply of course to the Cebus monkey.

It is impossible, with the rough standard of comparison available at present, to eliminate entirely the possibility that one or more chromosome pairs may have been duplicated in those animals with more than 48 elements. That is to say, that we may have present in these cells four homologous chromosomes (of certain chromosomes) rather than the normal two. It is well known that occasionally in maturation a chromosome pair may fail to separate (non-disjunction) so that the resulting gamete may carry more than its normal complement of chromatin. Were we to assume that this process had taken place a number of times in the horse, the high number of this animal could be accounted for. However, since fragmentation is indicated by cytological observations and is adequate to account for the facts, we need not consider the remote possibility that duplication of chromosomes is responsible for the condition encountered.

The occurrence in some forms of a smaller number of chromosomes than 48 is also open to two interpretations; either there has been an end to end fusion of certain elements or else whole chromosomes have been lost. A comparison of the rabbit's chromosomes with those of man will show that in the rabbit we do not have as many small elements as in animals with 48 chromosomes. The condition of the rabbit may be satisfactorily explained by assuming an end to end fusion of some of the autosome pairs, thereby reducing the total number to 44. This seems more plausible than that two chromosome pairs were entirely lost, though the latter should be borne in

mind as a possibility and eventually tested out genetically.

Both the processes of end to end fusion and of fragmentation of chromosomes have been reported for closely related species of animals and plants. Fragmentation has figured most prominently, perhaps, in plants where it is especially common in the Liliaceae. Hance ('18) has worked out with thoroughness the case of *Oenothera scintillans,* where a fragmentation of chromosomes may occur in different individuals of the same species. End to end fusion, on the other hand, is especially well seen in the Diptera, as the work of Metz ('14) has shown. In the eutherian mammals the evidence indicates that both fragmentation and end to end fusion of chromosomes have played rôles in producing derivatives from the primitive complex.

As a general conclusion from the facts and considerations presented above, it is apparent that the chromosome constitution of the eutheria is closely similar. 48 seems to be the typical and probably the basal number for this group, and derivatives from this have been due either to fragmentation or to end to end fusion. There is no evidence which indicates that there has been any great change in the total amount of chromatin, either by additions or by losses. There is, furthermore, no evidence that polyploidy has played a rôle in species formation.

It is clearly recognized by geneticists and by cytologists that neither a fragmentation of the chromosomes nor an end to end fusion of different elements would exert any influence on the evolution of a group. Fragmentation would tend to decrease, somewhat, linkage, and bring about thereby a greater random assortment of genes, for when a chromosome breaks in two, genes located on either side of the break would be separated and the linkage formerly present would be destroyed. End to end fusion, on the other hand, would have the opposite effect, of course; at the point where chromosomes fused genes would become closely linked, whereas before they enjoyed

a random assortment. The individual genes, however, would not be changed by these associations.

The marsupials have a general uniformity in their chromosome complex which is strikingly different from that found in the eutheria. Yet according to our phylogenetic conceptions the ancestors of the marsupials and the eutheria must have come from a common stem and must have possessed at one time the same chromosome constitution. This brings us to a consideration of two questions; first, what was the primitive chromosome constitution of this common ancestor, and second, what relation do the chromosomes of the eutheria bear to those of the marsupials.

The evidence which we have of the chromosome constitution of the vertebrates below the marsupial level is too fragmentary to allow us to form a reliable judgment. We know nothing about the chromosomes of the monotremes, and in the reptiles only the Lacertilia have been carefully studied.

In the lizards it is clear both from my own work (Painter '21) and that of Dalcq ('21) that we have the bulk of the chromatin localized in a small number of larger chromosomes, while a small portion of it may be scattered in a great number of dot-like micro-chromosomes. In the Iguanidae in which I have studied six species, the number of these large chromosomes is invariably 12. Fig. 6 is a spermatogonial plate of *Anolis carolinensis*. In the family Teiidae the number of the large chromosomes was higher, probably about 26. Dalcq found 19 large elements in *Anguis fragilis*.

The present-day amphibia have been extensively studied and the chromosome numbers reported have usually been 24 or 26. In *Rana temporaria*, which has recently been carefully studied by Witschi ('24), there is a tendency for the greater part of the chromatin to appear in a few large chromosomes (Fig. 7). This figure was copied from Fig. 3, Plate XIX of Witschi's paper.

If we were to generalize from the limited observations on the reptiles and amphibia, we would have to conclude

that in the lower vertebrates the chromatin tends to occur in a small number of large chromosomes. However, the Anura and the Lacertilia are such specialized offshoots from the amphibia and reptilia, respectively, that the value of evidence from either is very problematical. The presence of the dot-like micro-chromosomes in the lizards suggests that changes in their chromosome constitution are under way. For the present, then, this question of the primitive chromosome condition of vertebrates below the marsupial level must be kept open. Extremely valuable evidence on this point should be given by the monotremes and by Sphenedon. It is hoped that cytologists who can obtain this material will not be slow in determining the chromosome constitution of these forms, for with the evidence of the monotremes and of the primitive reptiles before us, we should be able to postulate, with reasonable assurance, the chromosome condition of the most primitive mammals.

Turning now to the second question of the relation between the chromosomes of the present-day eutheria and marsupials, the great differences which we observe in these two sub-classes may be explained in one of two ways. It has been suggested a number of different times that the high number encountered in some of the eutheria was caused by a doubling of the chromosome number (tetraploidy) at some point in their ancestry. This suggestion has been founded, in some cases, on erroneous observations. For a long time the available evidence seemed to indicate that 24 was probably the typical eutherian chromosome number, and when higher numbers were reported, for example, 36 or 48, they were regarded as triploid and tetraploid races, no account being taken of the difficulties which would arise due to the presence of sex chromosomes in mammals. Thus when d'Winiwarter ('12) reported 47 chromosomes for a white man, and 24 had been previously reported for the negro, it was suggested that these differences could be accounted for by a doubling of the autosome number in the white man. My work clearly demonstrated later that the chromo-

somes of the white and negro races are alike. Polyploidy, however, is still regarded as a possible source for differences in chromosome numbers in mammals by Gates ('23, page 19). The second possible way of explaining the observed differences between the marsupials and eutheria is that the total amount of chromatin is approximately alike in these two sub-classes, but that in one case it appears in large packets, so to speak, while in the other we have smaller aggregations. The observed facts accord better with this last possibility.

In the foregoing pages I have pointed out that from a theoretical point of view we should expect the elements which determined sex to be the most stable of the chromosomes, during the course of evolution, for the reason that any great change in the makeup of the X chromosome would doubtless result in sexually abnormal individuals. That this theoretical expectation is borne out by cytological observations is apparent from Fig. 8. In this figure we have the sex chromosomes of the opossum and of four eutherian species reproduced at approximately the same magnification. It will be noted that in all cases the type of sex chromosome is the same, and considering the difference in cell size, the size of the X chromosome is not very different in forms as far removed from one another as the opossum and man. It would seem therefore that the X chromosome would afford us a rough measure of chromosome volume which will be of value in estimating the quantity of chromatin in the autosomes of the opossum and the eutheria.

In Fig. 21 on Plate I we have the spermatogonial chromosomes of the opossum reproduced at the same magnification as the other forms. Using the X chromosome as a measure of chromosome size or volume, it is clear that most if not all of the chromosomes of the opossum are very much larger than any of the autosomes of the eutheria. If we could cut up the opossum chromosomes in some way, we would probably have enough chromatin to make the eutherian autosomes. We have no way of knowing at present which is the more primitive condition,

the high number found in eutheria or the low number characteristic of the opossum, but the figures make it abundantly clear that the total amount of chromatin found in animals at and above the marsupial level is not greatly different. Either fragmentation or end to end fusion, as the case may be, will adequately account for the observed chromosome constitution in marsupials and eutheria.

In the insects we do not always have a uniformity in size of the X chromosomes in related forms; in the Heteroptera the X may be very large as in Protenor, or small as in Largus (see Wilson '25, p. 755). It is obvious that we could not use the X element in the Heteroptera as a measure of chromosome size. This, however, does not alter the fact that in mammals the X is more or less constant in size and may be used as a rough standard of measure for the autosome. Just how the X chromosome in insects may vary greatly in dimensions without markedly affecting the somatic characters of the different species is not clear. It is possible that in some forms the X chromosome may carry in addition to the chromatin concerned with sex determination and sex-linked characters a considerable amount of inert chromatin (inert in the sense that it carries no genes) just as is the case with the Y chromosome in Drosophila. In the latter case the genetic evidence demonstrates that the Y chromosome is empty (of genes) although in its staining reaction it appears to behave like the other chromatin in the cell. If in some insects the X were freighted down with inert chromatin the loss of some of this would have no effect on either the sex or the somatic characters of the progeny.

I have pointed out in a recent paper (Painter '25) that the assumption that polyploidy has occurred in mammals is beset with difficulties because of the presence of sex chromosomes. There is no question but that the cytological basis for polyploidy exists in the testis of mammals and other vertebrates. Tetraploid germ cells and giant spermatozoa have been reported quite generally

for the vertebrates, and I have myself recorded their presence in lizards, in the opossum and in man. In Study II (p. 309) I pointed out some of the consequences which we would expect if a giant spermatozoon were to function in fertilization. We have no reason to believe that tetraploid germ cells are confined to the male sex. It has been generally assumed that giant spermatozoa, along with other atypical spermatozoa, were not functional, but there is no apparent reason why a giant egg cell could not develop after normal fertilization. However, in the end result, it would make little difference which sex contributed the gamete carrying double the normal number of chromosomes. With the doubling of the autosomes we would have a doubling of the sex chromosomes. If the doubling were to take place in the female, then the egg after maturation would carry $2n + 2X$ chromosomes, and upon fertilization with normal sperm we would have zygotes with the following constitution, $3n + 3X$, or $3n + 2X + Y$ (depending on which type of sperm, the X or the Y carrying individual, functioned). The $3n + 3X$ individuals would be triploid females, and the $3n + 2X + Y$ individuals would probably be intersexes, if we may judge from the work of Bridges ('22, '25).

If the doubling took place in the male germ cell, we would have on fertilization with a normal egg triploid individuals with the following possible sex chromosome constitutions: $3n + 3X$, $3n + 2X + Y$, and $3n + X + 2Y$.

The further reproduction of triploid individuals with normal stock would result in a variety of different possible zygotic combinations of sex chromosomes. Bridges ('25) in a paper which has just appeared has enumerated the expected classes and indicated the sexual character of those which have been observed in Drosophila. As he has shown, tetraploid individuals may arise (females) but, even were such individuals viable, it would require rigid selection and matings to maintain a tetraploid race.

There is one additional reason why tetraploid individuals could not be maintained in mammals. During the past three years I have followed the behavior of the tetra-

ploid germ cells of man in maturation. The point of significant interest is that during the growth period of primary spermocytes, no chromosome-nucleoli appear in tetraploid cells. Normally the chromosome-nucleolus is made up of the sex chromosome elements (Painter '24a) which retain a condensed form during this phase of spermatogenesis. The absence of chromosome-nucleoli is interpreted to mean that when two X chromosomes are present in a cell, they undergo synapsis, and behave otherwise as the autosomes do. The same thing happens apparently to the Y chromosome. We should expect that in the maturation divisions the sex chromosomes (X and Y) pairs would act independently and would be distributed as in the egg. If this were the case, each sperm would carry both an X and a Y chromosome. On fertilization with a normal egg, only one type of zygote would appear $2n + X + Y + n + X = 3n + 2X + Y$.

We may conclude, therefore, that extensive polyploidy could not have played a rôle in animals at and above the marsupial level.

In the present paper a good deal of attention has been given to chromosome numbers, yet at the same time it has been fully realized that numbers *per se* are of secondary importance. The significant point is that as far as we can gauge it, the total amount of chromatin in the different mammalian groups is about the same, and there has been a remarkable stability in the chromosome associations. Inferentially, we may surmise that the total number of genes is about the same in all groups. In their chromosome constitution the mammals have shown themselves, so far at least, comparable to an order of insects.

If my general conclusion is a valid one, then we may expect that the plotting of chromosome maps in the eutheria will go forward with comparative rapidity, because linkage values established in one group or species can be applied to other forms. For example, if genes a, b and c are located in one chromosome in the rabbit,

and genes c, d and e are found in the rat, we should expect, from chromosome studies, that when genes a and b are eventually found in the rat, they will have the same position as in the rabbit. Transverse fragmentation or end to end fusion will occasionally upset these relations, but on the whole they should prove the same in different forms, and enable us eventually to plot the chromosome maps of the eutheria, although not all the characters will have been observed in one species.

Up to the present time my work has led me to make as broad a survey of the eutheria as possible. The next logical step is the intensive study of the chromosomes of different species within an order, to determine the limits to which fragmentation or end to end fusion has been carried within a small group. For this purpose I have chosen the rodents, both because of the large number of different species readily available for study, and because these animals have been used so extensively and will allow us to test out genetically the validity of the conclusions to which our cytological studies have brought us.

At the present time a number of the common rodents are being studied at this laboratory, but a host of other species not available to us in Texas can be readily obtained and studied at other universities in this country. With the technique I have used in my studies, the preservation of mammalian tissue presents no great difficulty and making chromosome counts becomes a matter of care and patience. It is hoped that other cytologists will become interested in the rodents so that we may have an extensive knowledge of the chromosomes within this group.

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THE REDUCTION DIVISION IN RELATION TO MUTATION IN PLANTS AND ANIMALS[1]

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THE reduction division has been the subject of much study on the part of geneticists and morphologists, for it constitutes one of those critical phases which are of fundamental importance in connection with biological theories, particularly those having to do with heredity. The meiotic or reduction mitoses are much easier to study in plants than in animals and are moreover much more constant in time of appearance. As a consequence of this situation, an enormous amount of information has accumulated on the botanical side regarding peculiarities of the maturation or reduction divisions particularly in relation to hybridism. It will be shown in the present article that the reduction division in plants and animals which have been used in recent years, as examples of the origin of species by mutation, is often abnormal and in essential features duplicates the peculiarities found in the meiotic mitoses of known hybrids.

To Rosenberg belongs the credit of having first clearly called attention to the meiotic peculiarities of hybrids in plants. His study of the cytology of reduction in a hybrid between the long-leafed and the round-leafed sundew, *Drosera longifolia* and *Drosera rotundifolia,* is now a classic. This sterile hybrid is not uncommon in northern Europe and passes variously under the names *D. ovata* or *D. anglica.* In this hybrid there is a very interesting situation present in the reduction division. As Rosenberg pointed out, the metaphase of mitosis in the heterotypic division is characterized by the presence of

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

ten double (bivalent) and ten single (univalent) chromosomes. According to Rosenberg, the ten double chromosomes represent the ten gametophytic chromosomes of *D. rotundifoia* associated with ten of the twenty gametophytic chromosomes of *Drosera longifolia.* The ten univalent chromosomes represent the surplus chromosomes from the *D. longifolia.* Further striking peculiarities of mitosis in this classic example are the lagging of the chromosomes in their movement towards the equatorial plane (metaphase) and a further lagging in their progress towards the poles (anaphase). This lagging is characteristic of the smaller or univalent chromosomes. Another interesting feature found in the Drosera hybrid is

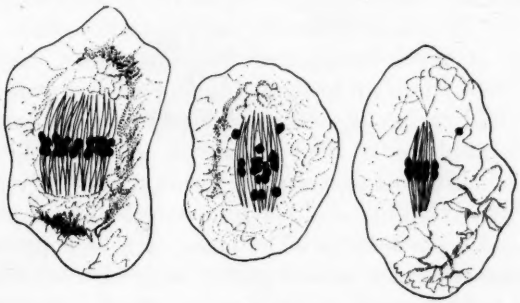

FIG. 1

the fact that from few to several of the univalent chromosomes are so tardy in their movement that they are frequently left out of the daughter nuclei. The result of this situation is the formation of small subsidiary nuclei. The final consequence may be the formation of more than the normal four pollen grains from each mother cell. The additional grains are smaller in size and owe their nuclear equipment to the lagging chromosomes.

Since the appearance of Rosenberg's epochal contribution to the cytology of hybrids, many publications have recorded similar phenomena in other known hybrids. In a summary article such as the present, it will be unnecessary to indicate these in detail, particularly as an admirable résumé of the literature has recently appeared in

Tischler's "Allegemeine Pflanzenkaryologie," Berlin, Verlag von Gebrueder Borntraeger, 1922.

It will serve a useful purpose at the present stage to indicate, in a general way, studies which have been carried on in recent years on the cytology of extremely variable large genera of plants. Rosenberg's work on the genus Hieracium is of fundamental value in this connection. He has shown that the species of Hieracium are characterized by different equipments of chromosomes and that these chromosomes behave in the fashion described above in the case of *Drosera ovata,* a hybrid between *D. rotundifolia* and *D. longifolia.* A very extensive and thorough investigation in this direction is furnished by Taeckholm's work on the European species of Rosa.[2] It has been shown here that the genus Rosa, although it has normally seven chromosomes, may have multiples of these up to eight fold; in other words, there are diploid, triploid, tetraploid, pentaploid, hexaploid and octoploid roses. The roses with the multiple equipments of chromosomes usually present not only a high degree of variability and sterility but also the characteristic meiotic phenomena of hybrids, such as supernumerary nuclei abortive pollen grains, lagging chromosomes both in metaphase and anaphase, bivalent and univalent chromosomes, etc., etc. Blackburn and Harrison have likewise carried on investigations on the roses with essentially the same result as those of Taeckholm and Rosenberg. In my own laboratory, Dr. A. E. Longley has investigated the cytology of numerous species of Rubus (blackberries) and Crataegus (hawthorn), with conclusion that many of these represent natural hybrids on the basis of their meiotic phenomena which closely resemble those found in the hybrid *Drosera ovata.*

It will be convenient in this connection to call attention to the meiotic phenomena in a cryptogamic hybrid. Not uncommonly in clear lakes one finds gigantic and sterile

[2] *Zytologische Studien ueber die Gattung Rosa,* von Gunnar Taeckholm, Uppsala, 1922.

examples of the genus Isoetes, the quillwort. These gigantic forms have many more leaves than the normal species and are much more vigorous vegetatively, often exceeding in dimensions three or four times those found in the parent forms. Fig. 2A represents the late meta-

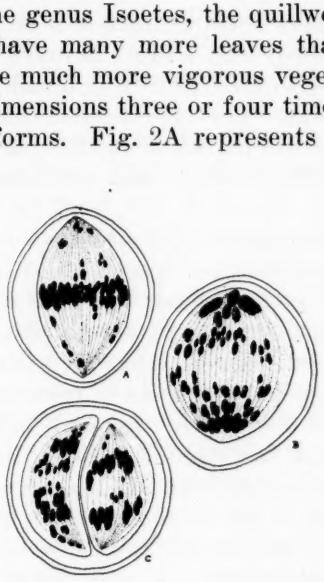

FIG. 2

phase of the heterotypic division in a hybrid species of Isoetes found in the Lakes of Law, Cape Breton Island, Canada. Fig. 2B shows the early anaphase in the heterotypic division. Fig. 2C shows the homotypic mitoses. In the item on the right of C is shown the early anaphase, whilst on the left is represented a later stage of the anaphase. In all the cells shown in Fig. 2 are represented conditions such as are characteristic of known hybrids. These are respectively the occurrence of two categories of chromosomes, large and small, and the marked lagging of the chromosomes, particularly those which are smaller in size, both in the metaphase and the anaphase, and in both the heterotypic and the homotypic mitoses. These gigantic forms of Isoetes as represented by the particular form under discussion are extremely sterile and the microspores are frequently entirely abortive, whilst the megaspores vary greatly in size and present striking ab-

normalities. This hybrid, which will be fully described elsewhere, is apparently a cross between *Isoetes echinospora* and *Isoetes lacustris,* as it is found associated with these two species. The gigantic individuals always resemble the lacustris parent, since they possess its characteristic habit, stiff texture and reticulately sculptured megaspores.

With these preliminary statements in regard to known hybrids and polymorphic species which resemble in all their essential cytological features hybrid forms, we may pass to the consideration of certain so-called mutating types. The genus Oenothera has been put forward as an example of the origin of species by mutation by the Dutch physiologist, de Vries. He observed that in cultures of *O. lamarckiana,* a very small fraction of the seedlings differed from the parent form. The various aberrant types originating in this way were sometimes very sterile and always more or less sterile as is also the case with the parental *O. lamarckiana.* It was found that these abnormal derivatives bred true to type and they were considered by de Vries as a consequence to represent elementary species or mutants. It has been generally conceived by those who adopt the doctrine of mutation that these elementary species are the result of a spontaneous change in the germ plasm of *O. lamarckiana.* It was found subsequently that other species of Oenothera had the same peculiarities as *O. lamarckiana* and in some of the American species the production of aberrant mutants is at times very large, amounting in some instances to 90 per cent. of the seedlings. Bartlett has proposed for this marked variability the phrase "mass mutation." In an interesting series of investigations, Davis[3] has shown that it is possible to produce artificially and by hybridization an Oenothera closely resembling *O. lamarckiana* to which he has given the name *O. neo-lamarckiana.* This interesting artificial hybrid resembles *O. lamarckiana* in

[3] "The Behavior of Oenothera neo-lamarckiana in selfed line through seven generations," B. M. Davis, Proceedings of the American Philosophical Society, Vol. 63, No. 3, 1924.

breeding relatively true, generation after generation, and also in throwing in cultures a relatively small number of elementary "species" or "mutants." There has been during the past two decades an enormous amount of activity in the investigation of the cytology and genetics of species of Oenothera. It is very generally agreed at the present time by geneticists of prominence that *O. lamarckiana* and other so-called mutating species of Oenothera are of hybrid origin. This conclusion is largely confirmed by the cytological conditions found in the heterotypic meiosis.

It will now be convenient to take an example from the cryptogams illustrating the process of mutation. Many years ago in a greenhouse in Cambridge, Massachusetts, there originated by chance a fern which is now known as the Boston fern. This is a derivative of the West Indian species *Nephrolepis exaltata* as shown by the fact that it originated in the same greenhouse with it. The Boston fern is very generally regarded by mutationists as a mutant of *N. exaltata.* This fern is distinguished by two features: first, by its almost complete sterility and second by its great vegetative vigor and adaptability to unfavorable conditions. The second feature has made it very popular as a decorative and a house plant. This fern shows a high degree clonal variation and the clones have originated from time to time a huge number of varieties, which have been exhaustively studied by Benedict,[4] who has put forward the view that the Boston fern exemplifies the origin of species by mutation. In one of the clones of the Boston fern known as variety *fertilis,* a certain number of spores are capable of germination and it has been recently shown by Benedict that these spores give rise to a very varied offspring in contrast to the parent species *N. exaltata* in which the sporelings are very uniform.

The great vegetative vigor and hardiness of the Boston fern as compared with its tropical progenitor, its ex-

[4] *Journal of Heredity,* 1924, and list of literature.

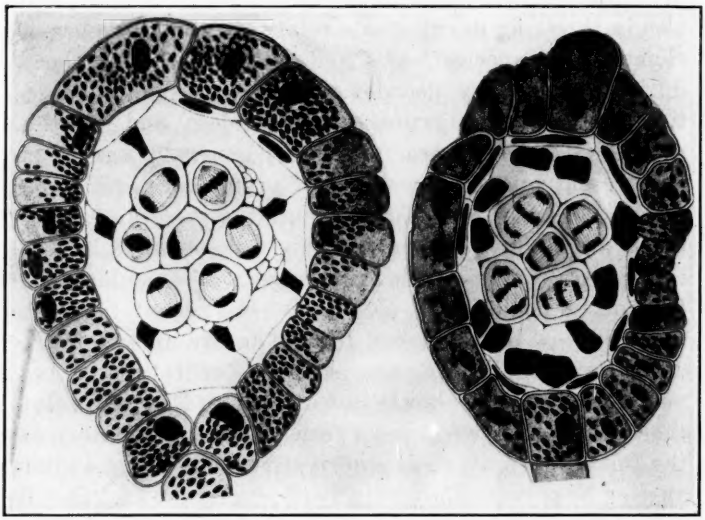

FIG. 3

treme variability, both in clones and sporelings, and finally its marked sterility are all indicative of a hybrid origin. The detailed cytological investigation of this polymorphic species will be described elsewhere, but it will be of interest in the present connection to introduce illustrations of the heterotypic mitosis in the parent *N. exaltata* and *N. exaltata* variety *bostoniensis*. Fig. 3A represents a sporangium of *N. exaltata* in which the spore mother cells are undergoing the first or heterotypic division. The illustration is on such a scale that it will be possible for the reader to recognize that the maturation divisions, both in the metaphase and anaphase, are entirely normal. *N. exaltata,* even when grown in greenhouses, produces a large number of spores which are apparently entirely normal in structure. Figure 3B illustrates sporangium of the Boston fern in the same stage of development, from a plant grown in the same greenhouse and on the same bench as the *N. exaltata* figured above. It will be obvious on inspection that the reduction or meiotic division in this sporangium is quite

abnormal and is characterized by the presence of lagging chromosomes such as make their appearance in known hybrids. The sporangium, moreover, appears somewhat shrivelled in contrast to the rotund outline shown in the previous figure. This shrinkage is a precursor of later abortive development, for normally in the fully developed sporangia there are no spores capable of germination, since all are shrivelled and usually without any protoplasmic contents.

It is thus obvious that the Boston fern has not only all the vegetative characteristics of a hybrid, such as extreme vigor and reproductive sterility, as well as great variability (in all these features it presents a marked contrast to the parental *N. exaltata*); but also the detailed investigation of the crucial meiotic phenomena in this fern indicates very clearly a hybrid (heterozygous) origin. It will thus appear, to all but those extremists, whose minds are blind to all but experimental evidence, that the Boston fern is a hybrid derivative of the tropical *N. exaltata.* It is not difficult to imagine such a mode of origin because hybrid ferns originate very frequently in greenhouses where a number of species of ferns are cultivated together.

It will now serve a useful purpose to call attention to the meiotic phenomena of insects, a group of animals very closely connected in their evolution with that of the higher plants. Fig. 4 shows the metaphase of the meiotic mitoses of a number of insects as taken from the recent third edition of Wilson's classic, "The Cell in Development and Heredity." A, B and C represent the spermatocytic divisions of three genera of beetles, namely, Trirhabda, Chrysochus and Photinus. D, E, F and G show the same condition in four Hemiptera, namely, Protenor, Leptoglossus, Anasa and Pachylis. Obviously in all these heterotypic metaphases designated above, the chromosomes are arranged in pairs end to end (telosynapsis) and exactly in the equatorial plane. In other words, the illustrations picked at random from Professor Wilson's admirable work show that the normal conditions in the

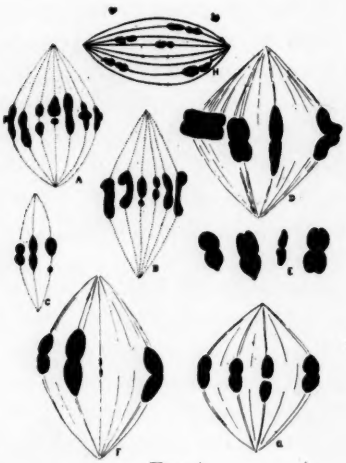

FIG. 4

critical or maturation mitoses in insects exactly resemble the corresponding conditions in the flowering plants.

With this preliminary statement, we may now pass to the consideration of the Pomace fly, *Drosophila melanogaster*. This dipterous insect has been the zoological counterpart of *Oenothera lamarckiana*, and a whole school of biology has practically been founded on the study of this extremely variable type. The discovery of its variability is apparently to be credited to Professor Castle,[5] of Harvard University. Later, Professor Morgan, of Columbia University, exploited the species in connection with the theory of mutation on the zoological side and a tremendous amount of activity has developed, particularly in genetical laboratories, in the direction of a study of the chromosomes and genetical characters of the Pomace fly. In all contemporary genetical textbooks it occupies a prominent position and is considered to exemplify and prove the origin of species by mutation. This situation is all the more remarkable because Oenothera has now passed for the most part into the biological discard, and very few, even among the geneticists, consider

[5] *Journal of Experimental Zoology*, 1906.

its peculiarities to be due to anything else but a hybrid origin.

A noteworthy feature of the cytological study of Drosophila, a genus to which the greatest importance is attached by mutationists, is our almost complete ignorance in the past of the all-important phases of the meiotic mitoses. It is true that Miss Stevens has given some attention to this subject, but, as was pointed out later by Metz, her methods were crude and unreliable. As a consequence, we have, up to the present time, no accurate information regarding meiotic or reduction divisions in *Drosophila melanogaster*. This is a surprsing condition in view of the great theoretical burden which has been put upon the chromosome apparatus in Drosophila. Morgan, in his recent summary of "The Genetics of Drosophila" (Bibliographia Genetica Deel II, 'S-Gravenhage, 1925) notes the difficulties connected with the study and interpretation of the meiotic or reduction divisions in this species. He states that they are both hard to investigate and to interpret.

Investigations carried on by the present authors seem to indicate that the employment of rapidly penetrating preservatives simplifies greatly the technical difficulties connected with the study of meiosis in Drosophila. The use of Carnoy's absolute alcohol, chloroform and acetic acid, the penetration of which is facilitated by a powerful air pump, brings about a very satisfactory general condition of preservation. This reagent is particularly valuable on account of its solvent action on the fat which is present in such large quantities in the body cavity of the pupae and which often constitutes an envelope surrounding the reproductive organs and shutting them off from the rapid action of preservatives. The fat-dissolving chloroform in Carnoy's solution successfully overcomes this obstacle to penetration.

The cytological investigation of the pupae, which are most satisfactory for the study of the meiotic phenomena in the testes, has made clear that the reduction division in *Drosophila melanogaster* presents the identical pecu-

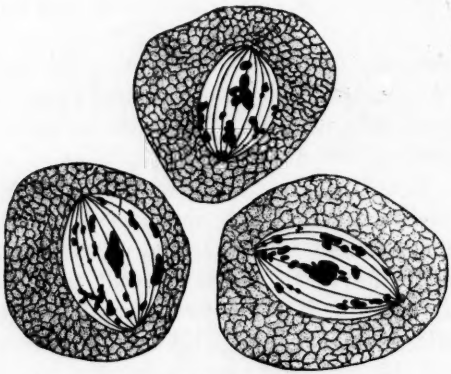

FIG. 5

liarities which have been noted above as characteristics of hybrids. The failure of Morgan and his associates to interpret properly the phenomena of meiosis in *Drosophila melanogaster* is in all probability due to their unfamiliarity with the literature dealing with the cytology of hybrids, particularly that of hybrid plants which is very large and important. Fig. 5 shows the formation of the spindle in the meiotic mitosis of Drosophila as exemplified by a large number of preparations which have passed under our observation. It is obvious that the chromosomes are irregularly distributed, after the manner of hybrids. Certain of the larger ones occupy a position near the equator of the spindle, whilst numerous smaller ones are distributed towards the poles. We have here a condition which corresponds to the early metaphase in divisions in normal species of insects. The irregular distribution of the chromosomes and their very large number are noteworthy features. By mass methods of sectioning, we have secured quantities of the relatively rare mitoses in the testes of Drosophila (wild, scarlet, spineless, brown and white). Later, the chromosomes tend to group in twos, and a notable number of them, especially in the later stages of mitosis, are cast out of the protoplasm. A more advanced metaphase is

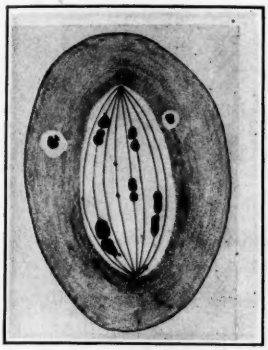

FIG. 6

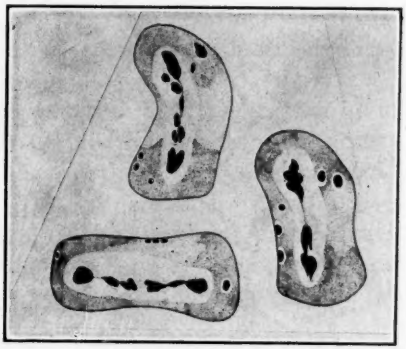

FIG. 7

shown in Fig. 6. Here the chromosomes are clearly paired end to end and lie along the threads of the spindle. We have obviously here to do with the synaptic stage of the metaphase. An interesting fact, however, is the failure of the synaptic mates to arrive simultaneously in the equatorial plane. We have never observed in the investigation of many thousands of preparations a single equatorial plate in which the synaptic mates are grouped in the normal fashion for insects. The conditions in fact, on the contrary, are distinctly those found in hybrids and certainly even if this interpretation is not accepted, the conduct of the chromosomes in meiosis is highly abnormal. Not only are the chromosomes abnormal in their conduct in Drosophila, but in the metaphase, particularly in its earlier conditions, are far more numerous than would correspond to a theoretical statement of Professor Morgan and his associates. They assert that there are four chromosomes, haploid, and eight chromosomes, diploid, in *Drosophila melanogaster*.

The anaphase of mitosis in the meiotic division of Drosophila is equally interesting. In Fig. 7 are shown a number of spermatocytes which are in this stage. The reader will observe that there are approximately eight chromosomes in the region of the spindle and that these tend to form two polar groups of four each. The illustra-

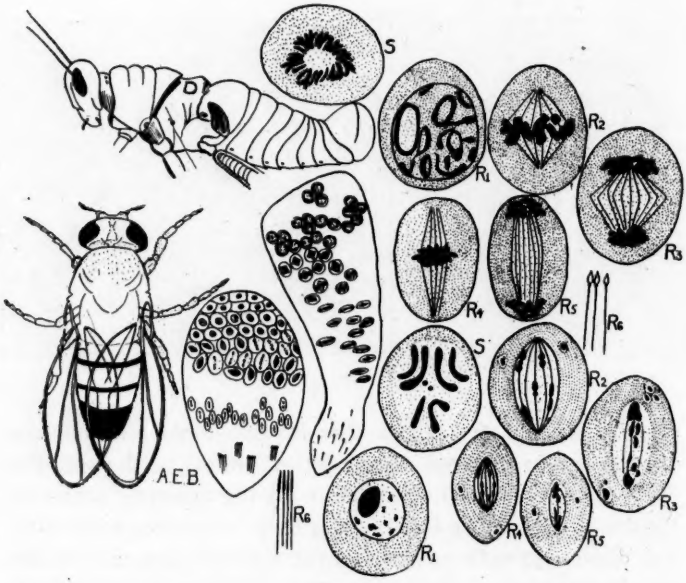

FIG. 8

tion shows a more regular condition than that which frequently occurs. Very generally the larger chromosomes reach the poles in groups of approximately four, leaving behind them large numbers of chromosomes of much smaller size in the intervening region. Another feature of interest in the anaphase is the passage of a larger or smaller number of chromosomes into the protoplasm also shown in Fig. 7.

It will be obvious to the reader who has followed the statements and illustrations appearing in previous pages that both the metaphase and anaphase of the meiotic mitosis (reduction or maturation division) in *Drosophila melanogaster* are distinctly of the type found in hybrids. Even if extreme mutationsists should refuse to grant this resemblance to be of significance, it is quite obvious that the highly important meiotic phenomena in Drosophila are entirely abnormal and aberrant. These abnormalities may be catalogued as follows: Polyploidy or unex-

pectedly numerous chromosomes, lagging of the chromosomes, absence of an equatorial plate and finally the elimination of many of the chromosomes in the later anaphase into the protoplasm of the cell.

If one adopts the comparative attitude in regard to these phenomena, one is obliged to consider at the very least the strong probability of the hybrid origin of *Drosophila melanogaster*. The mitoses in its meiotic divisions resemble, for example, quite strikingly those in such a well-known hybrid as the mule, and have their abundant counterpart in the very numerous data presented by the cytological phenomena of hybridism on the plant side. Of course, if one takes the point of view that the comparative and structural study of plants and animals is out of date, these facts would have no significance except as abnormalities. It appears pertinent, in this connection, to bring into court the close relationship between chemistry and physics. The physicist, for example, who attempted to draw any serious conclusions from the spectroscopic study of material, which had not been passed upon previously by the chemist, would be open to the serious charge of inaccuracy and superficiality. Similarly, it appears obvious that the physiologist or the geneticist who disregards the fundamental structural characteristics of the material with which he works is likewise open to severe criticism. Not a few of our physiologists and geneticists at the present time seem to merit the description aptly bestowed by Mr. Kipling on the personages of the psychological novel, "They steam for steaming's sake . . . their port is all to make."

If one takes the attitude that only experimenal data are of any importance in biological investigations, the point of view apparently adopted by many extreme experimentalists, the result will inevitably be extreme sterility in the biological field. Living beings are distinguished, above all, from inanimate matter by the capacity for reproduction and inheritance. These distinctive phenomena are largely connected with structures and ob-

viously structures can not be left out of sight in any theory of inheritance no matter how well founded experimentally. It is clear, however, that mutationists of the Morgan school have not rejected structures in the study of heredity, since they attach the greatest importance to the chromosomes and their significance as the bearers of hereditary characteristics.

If we follow the geneticists of the Morgan school in evaluating chromosomes as of high importance, and if we go further than the members of this school have yet gone in bringing into court also the crucial and critical meiotic phenomena which they have practically entirely neglected, we are compelled to conclude that the Pomace fly is thoroughly abnormal in its maturation or reduction divisions and that the abnormalities present a detailed and striking resemblance to those presented by known hybrids. The only reasonable concluson from this situation is that *Drosophila melanogaster* is a hybrid and that both its extreme variability and its remarkable meiotic divisions are the result of this fact. It follows, of course, that the huge and top-heavy superstructure which has been erected by the extreme school of geneticists on the too fragile basis provided by the Pomace fly falls of its own weight. This is not surprising, as similar course of events has already presented itself in the pendant case of so-called mutation on the plant side, namely, *Oenothera lamarckiana.*

It will serve a useful purpose to draw a clear parallel between the divisions of the cells in the spermatogenesis of such a type as the grasshopper, which is commonly used in the laboratory for illustrating the general principles exemplified by the mitotic divisions in the spermmother cells of animals, and those found in the Pomace fly, which has been used as the basis of a theory of evolution by so-called mutation. Fig. 8 in its upper part shows a diagrammatic illustration of a male grasshopper. Above the insect is represented one of the somatic cells with its large equipment of chromosomes. To the

right, R. 1, it shows the spermatocyte getting ready for its first division and exhibiting a notable number of the ring chromosomes found in the Orthoptera, etc. R. 2 shows the metaphase of the first division of the spermatocyte. R. 3 illustrates the anaphase of the same division. It will be noted that the chromosomes are quickly and sharply transferred to the equator and to the poles, respectively, in the metaphase and anaphase. R. 4 and R. 5 show the corresponding phases of the second division of the spermatocytes, and R. 6 illustrates on a smaller scale of magnification three spermatozoids, such as result from the final divisions of the spermatocytes. Below the figure of the grasshopper and to the left of the spermatocytic divisions described above is shown a diagrammatic view of one of the segments of the testes of the grasshopper. In the upper region appear spermatocytes with ring chromosomes. Below these are spermatocytes in the first division. Lower still are found the second divisions of the spermatocytes and in the lower region of the lobule are represented fully developed spermatozoids.

The figure in the lower left-hand corner of the illustration represents a male Pomace fly (*Drosophila melanogaster*). To the right of it is shown a diagram of one of the testes. In this are exhibited the reproductive cells in the various stages of development corresponding to those of the grasshopper. Fig. S to the right of the testis of the grasshopper contains the well-known diagram of the chromosomes in the somatic cells of a male *Drosophila melanogaster*. R. 1 shows a spermatocyte in the developmental stage. In R. 2 is represented the metaphase of the first division of the spermatocyte. In R. 3 the anaphase of the same division is depicted. In both these figures it will be noticed in contrast to the situation in the grasshopper that there is no assemblage of the chromosomes in the equatorial region and at the poles in the definite manner found normally in dividing spermatocytes. It is further noted in R. 2 and R. 3 that there is an

extrusion of chromatin from the nucleus into the protoplasm. In R. 4 and R. 5 are shown the metaphase and anaphase, respectively, of the second spermatocytic division. In R. 6 appear the spermatozoids resulting from the second spermatocytic division.

The diagrammatic illustrations in Fig. 8 make it clear that the spermatocytic or meiotic divisions of *Drosophila melanogaster* are fundamentally different from the normal conditions found in the corresponding cells of the grasshopper. It has been shown above that the geneticists, who have made a special fetish of Drosophila, have failed to notice this abnormality in the meiotic phenomena. Having failed to notice the aberrant maturation, they have naturally failed to offer any interpretation of the peculiar conditions present. It is of importance to note that the meiotic mitoses of the Pomace fly present a striking and inescapable resemblance to the conditions which present themselves in the reproductive cells of plant and animal hybrids. It follows that the theoretical conclusions drawn from the exhaustive study of *Drosophila melanogaster* must be fundamentally revised. *Drosophila melanogaster* obviously presents an example of a phenomenon which is extremely common on the plant side, namely, the appearance of a natural hybrid. The evidence grows continually that a large number of what are called species in plants are not pure-blooded forms but hybrids.

THE OCCURRENCE OF AN EYE AND OF A TOOTH ABNORMALITY IN A LINE OF ALBINO RATS

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IT has been demonstrated many times, in those forms whose eggs are fertilized outside the body cavity of the female, that deviations from the normal oxygen supply are capable of producing metabolic changes in the developing embryo. Since such changes result in visible disturbances of the growth of certain organs, one is led to believe that their effect might be more fundamental than the observed external anomalies, and that the germ plasm might also be influenced. This experiment was undertaken with the dual idea of attempting, by this method, to alter the developmental rate of mammalian embryos directly, and to discover if, at the same time, a more fundamental metabolic change were produced which might affect the germ cells.

The experimental procedure is given, and the behavior of the stock recorded. It should be stated at the outset that no cause and effect relationship is claimed between the experimental procedure and the results obtained. These results are given in order that other investigators may know of their occurrence. The experimental method is explained with the idea of adding another possible approach to the problem of experimental modification of the germ plasm.

The experimental production of abnormalities in those forms whose eggs are either laid or developed outside the body of the female has been a fertile field of investigation. Stockard (1921) reviewed the work which had been done up to that time from the viewpoint of developmental rate and structural expression. His theory to account for the abnormalities is based on the

fact that every individual varies slightly from every other individual due to differences in development. The variations are noticed when they appear in exaggerated form, and are then recognized as anomalies. The propositions advanced are: that all types of abnormals or monsters may be caused by the same experimental treatment; that any single type may be caused by a great number of different treatments; that all effective treatments tend primarily to lower the rate of development; and that the type of monster induced depends on the particular developmental moment or moments during which the developmental rate was reduced.

In a given species there is a specific rate of development at which the early cleavage stages follow one another, and at which the formation of different organs begins. This may be due to the chemical structure of the egg and to the oxidation rate of the material in the egg. Although in the later stages the amount of food available influences the rate of development, the supply of oxygen and the temperature have a more striking effect.

The two methods most commonly employed to induce alterations in the developmental rate are: temperature changes and oxygen deficiencies. No experiments with mammals are recorded in which these methods of directly influencing prenatal development were consciously employed.

The work with mammalian material has been done chiefly with the hope of affecting the germ plasm. The experiments of Stockard (1912, 1913) with alcohol on guinea pigs; of Guyer (1918, 1920) with antibodies in rabbits; of Little and Bagg (1923, 1924) with X-rays on mice; and of Hansen (1924) with alcohol and X-rays on rats may be mentioned here. In all these cases an outside agent, chemical or physical, was employed as an "activator." In undertaking the present investigation, the plan was to attempt an alteration in the normal rate of development of the embryo by some method which would cut off the oxygen supply at a critical time. It was

thought that this could be accomplished by delaying implantation. There was thus the possibility of obtaining two reactions: an external one, due to an upset in development; and an internal one, due to the absence of sufficient oxygen for normal growth, with the possible accumulation of carbon dioxide.

Stock sent us from the Wistar Institute as Line A Selection stock was employed.

Methods

In order to obtain an adequate control, in addition to the use of the Line A Selection rats, a litter of young was first obtained under normal conditions from a given male and female. These young were bred inter se and kept as a control line for comparison with subsequent litters from the same parents after treatment. After the weaning of this control litter, the female was examined daily by the smear method described by Long and Evans (1920) for determining stages in the oestrous cycle. When the smear indicated that the female was in heat, she was again placed with the male, and the mating observed. The female was then put in a cage by herself, under the same environmental conditions, and with the same food as the other rats of the colony.

Following the schedule worked out by Long and Evans (1920) for the position of the fertilized egg at different stages in its development, the fourth day after fertilization was selected as the most favorable time at which to attempt to produce some metabolic change which would affect the blastula. At this time, the fertilized egg has presumably reached the uterus, but has not yet become implanted. The initial oxygen supply of the egg may, therefore, have become nearly exhausted, and the means of reaching its next supply not yet established, since that is to come, after implantation, from the maternal blood. If at this stage, then, implantation could be delayed, the oxygen supply might be impaired, if not absolutely cut off, and an alteration in the metabolic rate induced. Sub-

sequent implantation might then occur, metabolic equilibrium be restored, and development continue.

OPERATIVE TECHNIQUE

Of the various methods tried at the beginning of the experiment, only one, that which was employed in the majority of cases, will be described here. The female was etherized, and a lateral, dorsal incision made. Without severing any of the connections, the ovary, Fallopian tube and part of the uterus were then lifted out of the body cavity, being held with forceps, and exposed to the air for five minutes during which they were cooled and dried by a gentle fanning. They were then replaced in the body cavity, and the incision closed with two or three stitches. In some cases, this operation was performed on the right side, in others on the left, and in a few animals on both sides. The rats recovered within a short time. They were closely watched for the next two or three weeks, and the effect of the operation noted. In some cases, pregnancy appeared to be interrupted, while in others it was apparently normal, and was terminated with normal parturition.

RESULTS

Twenty-seven females were treated experimentally. Six of these were etherized only, in order to test the effect, if any, of that part of the operative procedure. Of these, five produced normal litters, whose subsequent offspring were all normal; one produced no young. Six other females were subjected to more drastic types of operation. Only two of these produced offspring, all of which were normal and produced only normal young. Fifteen females were treated by the technique described above. Of these, ten produced offspring, and five failed to become pregnant.

Of the ten experimental litters, only one showed any abnormality in either the first (treated *in utero*) generation, or in several succeeding generations in which close

inbreeding was practiced. The one female (female 69) who was the mother of the first abnormal eyed individual, had the left ovary and tube exposed and fanned for five minutes. Three young were born twenty-two days after the observed mating, two of which, a male and a female, had abnormal eyes. Female 69 was mated twice after this litter to the same male (male 16) who had sired the first litter. Thirteen young were born in each litter, and one individual in each of these litters became abnormal in one eye during the course of its lifetime. They were not noticeably abnormal at birth, and certainly not at weaning. Later experience has shown us that there are often borderline cases, where the abnormality is so slight as to be barely discernible. Since there had been no opportunity to observe the degrees of the reduction in the size of the eyes at this time, it may have been that these individuals did have a slight variation at birth, which was not detected because of inexperience.

Female 69 was also mated with her abnormal son, male 99, producing four litters, totalling nineteen young. All these were normal. These lines continued for four generations and produced no abnormals.

From the three litters (including the one *in utero* at time of treatment) of female 69 by male 16, four lines have developed. Line I is from the mating of the first two abnormals inter se—female 101 by male 99. Three litters were produced, in the second of which the first two "tooth abnormals" occurred. Two subsequent generations were obtained from this litter, but no abnormals, either eye or tooth, developed. No young were obtained from the third of these litters. The first litter, however, was mated (brother by sister) and this line was carried to the sixth generation (from the treated parent). One hundred and eighty-six young were born, of which four had abnormal eyes. These were all in the same subbranch, one appearing in the fourth generation and three in the fifth.

Line II developed from the mating of abnormal female 101 by normal male 148, a sib in a later litter from female 69 by male 16. This branch was carried to the sixth gen-

eration by brother to sister matings. One hundred and fifty-seven young were born, one of which, a male, in the fifth generation, was abnormal (eye). Three litters, totalling eleven young (all normal) were obtained from him by his sister. A female from one of these litters was backcrossed to him, producing six young, two of which developed abnormal teeth. Four litters were obtained by brother to sister matings from this cross. Of these twenty-three individuals, eight have abnormal eyes. This line is being continued at the present time.

Line III was formed by brother to sister matings within the second litter obtained after treatment from female 69. This line was carried to the fourth generation with 126 young produced, and although one of the first generation females used for breeding was abnormal, no abnormal animals appeared in the progeny.

Line IV is the result of crossing the five females in the third post-operative litter from female 69 by male 16, with their sib (in a previous litter), abnormal male 99. Four of these females were normal. The branches from them were carried to the fourth generation with 112 young, one of which had abnormal eyes.

The fifth female in Line IV was herself abnormal eyed, the condition not becoming noticeable until she was adult. Like her normal sisters, she was also mated to abnormal male 99. Two litters of young were produced, totalling fifteen individuals. All were normal. The first litter was continued by brother to sister matings, giving rise to seven litters with twenty-seven young. In one of these litters, two abnormals developed—male 949, abnormal eye; and female 951, abnormal teeth. Three of the other litters were continued by the same type of mating, but among their seventy-eight offspring no abnormalities developed.

Male 949 was mated four times to his normal sister, female 950. Among their twenty offspring, two eye abnormalities were found. These occurred in different litters. Male 949 was also mated to his abnormal (tooth) sister, female 951. Twenty-seven young were born, one of which developed abnormal teeth. The litter mates of

this abnormal individual were inbred by brother to sister matings, producing eleven litters, totalling forty-three individuals, twelve of whom had abnormal eyes, and three of whom had abnormal teeth. It is worthy of note that four of these litters consisted of only two young, and in each case both of the young were abnormal in one or the other of these respects, and in one case in both respects. An effort was made to make as many abnormal by abnormal matings as possible among these individuals, regardless of whether the abnormals were litter sibs or not. Three such matings were made. One resulted in four young, two of which were abnormal; one in seven young, one of which was abnormal; and one in six young, one of which was abnormal. In one case, an abnormal-eyed female was backcrossed to abnormal male 949, producing four litters, totalling twenty young, six of which had abnormal eyes, and two of which had abnormal teeth. In two other cases normal females were backcrossed to abnormal male 949. These matings produced normal progeny. In one litter, a normal female was bred to her abnormal brother, producing four young, all of which were abnormal.

From three of these litters in which there were abnormals, the next generation has been obtained, twenty-nine young having been born. Thirteen of these are abnormal. This generation is not yet completed.

Sibs (but not litter mates) of the parents of abnormal male 949 produced five litters, totalling forty young, one of which was abnormal. This abnormal female was mated to male 949, but all their offspring were normal, and no abnormals developed in the next generation or in a backcross generation.

SUMMARY OF RESULTS

		Normal	Abnormal
Line I	F_2	24	2
	F_3	29	0
	$F_{3\,1\text{-}2}$	22	0
	F_4	73	1
	$F_{4\,1\text{-}2}$	14	0

Line I	F_2	24	2
	$F_{5\ 1-2}$	15	0
	F_6	16	0
		219	6
Line II	F_2	7	0
	F_3	60	0
	F_4	77	0
	F_5	45	1
	F_6	11	0
	$F_{6\ 1-2}$	4	2
	$F_{7\ 1-2}$	16	8
		220	11
Line III	F_2	73	1
	F_3	31	0
	F_4	21	0
		125	1
Line IV	F_2	58	1
	F_3	115	4
	F_4	124	3
	$F_{4\ 1-2}$	0	9
	F_5	43	15
	F_6	13	8
	$F_{6\ 1-2}$	27	6
	F_7	16	10
	$F_{7\ 1-2}$	10	3
		430	67

DISCUSSION

From the pedigree shown here, it is very evident that a complex situation is involved. In attempting a solution, it may be well to start with a process of elimination, and to consider first the various possibilities to which the observed conditions might be due:

(1) The abnormality might have been present in the stock before being subjected to experimental treatment. Since the rats, as stated, were Line A Selection Wistar stock, any such anomalies would have been noted in that stock at the Wistar. That there were none, Dr. King assures us. Furthermore, none were observed in the controls which were as closely inbred as the experimental lines.

(2) The abnormality may have been coincident in origin, but in no way dependent upon the treatment. The chances of such an occurrence are infinitely small, when chance alone is considered. However, this may have been the case.

(3) The remaining possibility is that the abnormality was produced by, or connected with the treatment.

The above seem to include the possible explanation of the *production* of the character.

The *nature* of the character may be considered next.

(1) It may be non-genetic. From a study of the pedigree charts this does not seem tenable. The stock of animals has been so closely inbred that the reaction of individuals from it to environmental conditions should be much the same. Furthermore, all the rats, control and experimental, were kept under exactly the same external conditions: they were fed the same kind of food, and were cared for in exactly the same manner. Were the effects, then, due to external environment, food conditions, or the like, the controls should show the same developmental responses. This has not been the case in a single instance.

(2) The character may be genetic. Considering it from a Mendelian point of view, it is clearly not a dominant trait. Neither is it a simple recessive—although in the broadest sense of the term—if the character is really Mendelian, it is a recessive under certain limited conditions.

It is not sex-linked.

In the preceding paragraphs, it is shown that we are not dealing with the ordinary types of characters. We do know, however, that some type of heredity or transmission is involved and that the method is extremely irregular. The stress up to this point in the experiment has been laid on obtaining a strain of true breeding abnormals. It was felt that until some conclusion in regard to the mode of expression of the abnormality was reached, it was useless to attempt outcrosses with unrelated strains. Therefore, no evidence can be offered at this

time that cytoplasmic inheritance is not involved. In order to obtain proof on this point it would be necessary, of course, to obtain inheritance of the character through a cross with an unrelated normal female. This will be attempted as soon as it seems feasible.

On the supposition that the character is inherited, there are several interesting hypotheses which may be advanced in partial explanation of the situation. The first of these is that the presence of normal overlaps is strongly indicated, since in case after case apparently normal individuals breed as abnormals. Moreover, the recorded amount of variation in the degree of the abnormality and the delay in its development as seen in two females (♀ 144 and ♀ X) where it appeared late in life, indicate a large percentage of overlaps with the normal condition. No effort has been made to calculate such a percentage, as it does not seem probable that normal overlaps alone are responsible for all the irregularities observed.

Another hypothesis which may be considered is that the character is dependent on many modifying factors. Since the pedigrees show that the percentage of abnormals is becoming greater in the later generations, it might be claimed that as the degree of homozygosity increases, the charcter is becoming less variable. Since the original stock was highly inbred, however, and since no outcrosses with the individuals not in the experimental line have been made, this does not seem to be a sufficient answer.

The situation is apparently too complicated to be explained either on the basis of normal overlaps or of increasing homozygosity of modifying factors. If the original abnormals—affected by the treatment while *in utero* —were due to a developmental retardation from a delay in implantation, or from a disturbance of oxygen equilibrium, some explanation must be given why the same type of anomaly appears as one of the heritable or transmissible characters; if they were induced by the treatment. It is well recognized that the eye region is one of the most susceptible portions of the embryo to any environmental

change, either internal or external. It is not necessary, then, to suppose that an explicit gene change has occurred or that any particular region or regions of the chromosomes, which might be responsible for the development of certain optic structures, has been affected. Under the circumstances, it seems much more probable that some metabolic or physiological condition, itself dependent on germinal constituents, has been altered, and may in turn be responsible for a delay in implantation in the embryos in each successive generation. Were such the case, either the maternal uterus, the embryo itself, or both, might be responsible for any delay which might occur. Furthermore, it is conceivable that the metabolism of either parent or embryo would vary within certain limits due to environmental fluctuations. Thus, although the germinal factors were present, they might not reach that stage of development which would cause an unfavorable physiological reaction. If, at the same time, a selective prenatal mortality of embryos were occurring, only those capable of surviving the altered internal conditions would come to birth. A second selection is also made in many cases on the basis of the breeding record. If no abnormals are produced after one or two generations from a given female, that line is discontinued. It may be that this line is potentially abnormal, but that the embryos survive the internal environment in better condition, and a larger percentage of normal overlaps is produced. Two kinds of selection are thus involved: one, due to an internal action which eliminates the grossly abnormal; and second, an artificial choosing, whenever possible, of those animals for breeding purposes of a recognizable degree of abnormality. Such an assumption would account for the larger number of abnormals occurring in the later generations.

It is interesting that the eye abnormality affects males and females alike, and apparently in equal numbers. The sex ratios of the treated and control lines are not significantly different.

Litter size shows a slight reduction in the experimental lines, falling to 5.34, while in the controls it is 6.20.

The eye and tooth abnormalities have appeared simultaneously in a sufficient number of cases to make it evident that they are not alternative expressions of the same causative agent—whether it be germinal or otherwise.

The discussion of the causes and nature of the characters here presented should not close without considering the possibility of an inherent tendency toward defects of development in this stock of rats. If there were such an innate condition toward lack of metabolic equilibrium in the stock which might have led eventually toward abnormal development without the intervention of outside factors, the experimental treatment may have had the effect of hastening the upset.

TOOTH ABNORMALITY

The tooth abnormality appeared for the first time in the second generation after the production of the eye abnormality in two individuals (a male and a female) from the same litter. It later appeared in female 951, and eight out of the ten tooth abnormals since that time have appeared among her direct descendants.

The condition is apparently the same as that described by Greenman and Duhring (1923) and by Hammett and Justice (1923). The incisors of either or both lower jaws become greatly elongated, some of them growing to form an almost perfect circle, the ends piercing through the maxilla or palatine bones. According to Greenman and Duhring, this growth is due to aberrant metabolic processes, the cases being less in number when a highly varied diet is supplied. Hammett and Justice state in their paper that the growth of the incisor teeth of normal albino rats is apparently continuous throughout life, and the reason that they do not reach a prodigious length is due largely to the fact that "an adequate apposition of upper and lower incisors ensures their grinding away their ends, as they are pushed out during growth. When such apposition is imperfect or lacking, the teeth become

abnormally long. This may be due to lack of opposing teeth, deviation of the jaws or prognathism." In the present case, it may be that it is due to a deviation of the jaws, since the skulls of the abnormal individuals show other developmental bone defects, especially in the region of the zygomatic arch. If, however, it is due to metabolic causes, since its expression is so closely limited to one branch within the experimental lines, it is not unlikely that its development depends on metabolic conditions which are an expression of a certain germinal constitution.

That it is not a localized failure of growth control accompanying old age is shown by the fact that it has developed in several individuals at the age of six or seven months, which is the height of their early maturity.

Relation of this Work to Other Investigations

If the abnormalities recorded here are the result of the experimental treatment, then another method of attempting to influence the germ plasm has resulted in an effect on the same region that has responded to various other types of experimental procedure. In view of the different opinions held on the specificity of response to treatment, the fact that another eye abnormality has occurred is of interest.

Summary and Conclusions

(1) An eye and a tooth abnormality which occurred in the Line A Selection stock of albino rats from the Wistar Institute are recorded.

(2) These abnormalities appeared after treatment in one experimental line, and none appeared in the controls. However, no claim is made that they have been *induced* by the treatment.

(3) The method of inheritance is irregular, the occurrence of normal overlaps complicating the situation.

(4) An inherited tendency toward delay in implantation of the fertilized egg is one of the suggestions offered as a possible cause of the eye abnormality.

(5) The experimental lines are being continued by close inbreeding in the hope of obtaining lines in which there is either no occurrence of normal overlaps or a predictable percentage of overlaps.

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THE RÔLE OF BUMBLEBEES IN THE POLLINATION OF CERTAIN CULTIVATED PLANTS[1]

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I. INTRODUCTION

ALTHOUGH bumblebees make very delicious honey, they usually store it in such small quantities, as compared with hive-bees, that it would not be practicable to keep them for this purpose. However, since Darwin (1841) came to their defense, eighty-four years ago, it has been shown that these industrious insects play an important and in some cases indispensable rôle in the pollination of many cultivated plants. While this fact, as we shall see later, is sometimes grossly exaggerated, there have been, on the other hand, several attempts to minimize or completely deny the economic importance of bumblebees.

The object of this paper is to record the results of some observations and experiments which throw additional light on this question. However, before taking up the discussion of this work, it seems desirable to review briefly the interesting "history of insect pollination."

The first one who clearly recognized the importance of insects in the pollination of flowering plants was the German biologist Kölreuter (1761).[2] About thirty years later, this important discovery was confirmed by Sprengel (1793) who, in addition to greatly extending the list of insect-pollinated plants, called attention to various remarkable floral structures and mechanisms and advanced

[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 236.

[2] Although it was known long before that the production of figs depends upon a small hymenopterous insect (*cf.* Hegardt, 1744; and Howard, 1899), it apparently did not occur to any of the earlier biologists that similar relations might exist between other insects and plants.

the theory—rather startling[3] for that time—that not only these, but the flowers themselves, are divine contrivances to insure insect pollination. Although some of his observations were later directly or indirectly confirmed, Sprengel's (1793) "theory of flowers" was rejected by most biologists (*cf.* Darwin, 1862; and Müller, 1873) and was in danger of being completely forgotten, when the Darwinian theory of evolution suddenly placed his observations in a new light. Inquiry into the relations between insects and flowers now became one of the most popular biological studies, and resulted in a phenomenal growth of this line of investigation, notably through the work of Darwin (1862 and 1876), Hildebrand (1865), Axell (1868), Delpino (1868–1869), Müller (1873 and 1881), Kerner (1876), Robertson (1888–1897), MacLeod (1893–1894) and Knuth (1898–1905). After a later brief period of comparative inactivity, the study of the ecology of insects and flowers has recently entered upon a new era which promises further important achievements for this branch of biology (*cf.* Knoll, 1921–1922; and Clements and Long, 1923).

II. Bumblebees and the Fertility of Red Clover

In Chapter III of his "Origin of Species," Darwin makes the following statement concerning the pollination of clover by bees:

> From experiments which I have tried [*cf.* Darwin (1858)], I have found that the visits of bees, if not indispensable, are at least highly beneficial to the fertilization of our clovers; but humble-bees alone visit the common red clover (*Trifolium pratense*), as other bees can not reach the nectar. Hence I have very little doubt, that if the whole genus of humble-bees became extinct or very rare in England . . . red clover would become very rare, or wholly disappear.

A few years later, this conjecture of Darwin (1859) was attacked in a series of papers by the American botanist, Thomas Meehan (1870, 1875, 1876a, 1876b), who as-

[3] In accordance with the anthropocentric viewpoint prevalent during the pre-Darwinian era, it was generally believed—even until quite recently (*cf.* Ogle, 1870)—that the fragrance and the various colors and forms of flowers were especially created for the purpose of pleasing the human senses.

serted that the fertility of clover is not dependent upon bees, or other insects, to any material extent.[4] Probably as a result of this adverse criticism, Darwin (1876) subjected this question to further experimental tests, and found that a hundred flower-heads of *Trifolium pratense* which he covered with a net did not produce a single seed, while a hundred others which were visited by bees yielded several thousand. But even this did not convince Meehan (1877a, 1877b, 1878, 1884), who now contended that Darwin's (1876, p. 361) results were due to differences in nutrition and not to insects. However, in 1885—three years after Darwin's death—there occurred an event, now well known to biologists, which convinced even such skeptics as Meehan (1893).

For many years the farmers of New Zealand, especially those of the South Island, had been obliged to import all their red clover seed, because of the fact that this important forage plant would not produce seed freely in their own country,[5] a condition which was changed almost over night by the successful introduction of bumblebees.[6] In addition to this important confirmation, Darwin's (1876) final conclusion regarding the relation of bumblebees to red clover is further directly or indirectly corroborated by the observations—and in some cases experiments—of Haberlandt (1880), Beal (1887), Pieters (1896), Kirchner (1905), Fruwirth (1906), Kuhnt (1908), Lindhard (1911 and 1921), Sladen (1912), Lovell (1918), Folsom (1922), Friese (1923), Pellett (1923) and others.

However, within the last few years, Darwin's (1859)

[4] Similar, though less sweeping, assertions were later made by Armstrong (1883) in regard to red clover in New Zealand, but this investigator seems to have placed too much faith in the rather superficial observations and experiments of Meehan (1875).

[5] This has been denied by Armstrong (1883), but his assertions are directly or indirectly contradicted by those of Dunning (1886), Thomson (1891) and Hopkins (1914).

[6] Not long ago, a similar lesson was very forcibly impressed upon some of our western fruit-growers who wasted large sums of money and much valuable time before they discovered that the Smyrna fig can not be profitably grown in this country without the insect (*Blastophaga grossorum* Gravenhorst) by which it is pollinated in the Old World (*cf.* Howard, 1899 and 1900).

position in regard to this question has again been assailed, this time by the Austrian biologist Heikertinger (1919), who bases his criticism on the following statement of Knuth (1898):

> Allerdings sind die Hummeln die hauptsächlichsten (nicht, wie Darwin meinte, die einzigen) Befruchter des roten Klees, doch bleiben nach Ausschluss derselben noch immer zahlreiche, normal saugende und Pollen sammelnde Insekten, um die zur vollen Fruchtbarkeit nötigen Fremdbestäubungen zu besorgen; es ist mithin das Glied der obigen Kette: "Je mehr Hummeln, desto fruchtbarer der rote Klee" unhaltbar.

In reply to this criticism, it may be stated, first of all, that both Knuth (1898) and Heikertinger (1919) have overlooked the fact that Darwin (1876) later subjected this question to further investigation, the results of which were published more than twenty years before the appearance of Knuth's (1898) criticism. In this report, Darwin (1876, p. 361) very clearly states his final conclusion concerning this matter in the following words: "It is at least certain that humble-bees are the chief fertilizers of the common red clover."

But even if we disregard this oversight on the part of Knuth (1898) and Heikertinger (1919), we are still confronted with the important question, whether or not it is true, as Knuth (p. 295) contends, that there are plenty of insects, besides bumblebees, which insure the complete fertility of red clover. While it is possible that Knuth's (1898) assertion may apply to a few restricted areas (*cf.* Knuth, 1897; and Franklin, 1912–1913, p. 203), there is, as we have seen, a large body of evidence which indicates that Darwin's (1859 and 1876) maxim, "the more bumblebees, the more fertile red clover," is probably true of most parts of the world.

In this connection, the following observations which the writer made in the vicinity of Boston from June, 1921, to November, 1924, seem worth recording. Throughout the summer and fall of these four years, large numbers of bumblebees could always be found working on red clover, unless the weather was very inclement. In addition to the bumblebees—of which fourteen species occur within the city limits of Boston—a female of some of the solitary

bees[7] and a few butterflies were occasionally found engaged in this work; but most of these visit the flowers in such a desultory fashion, as compared with bumblebees (*cf.* Clements and Long, p. 68), that the red clover in the vicinity of Boston would probably set little or no seed if its pollination depended upon other insects than bumblebees.

In regard to the hive-bee, concerning whose relation to red clover there has been considerable controversy (*cf.* Pellett, 1923), it may be stated that during the whole course of these observations not a single individual of *Apis mellifica* was seen on *Trifolium pratense,* although large numbers of them were working on alsike (*Trifolium hybridum*) and white clover (*Trifolium repens*) which was growing among or near the red clover. However, the writer does not wish to deny by this statement that *Apis mellifica* may occasionally obtain nectar and pollen from *Trifolium pratense.*

III. Experiments with the Hardy Garden Larkspur

Like red clover, the hardy garden larkspur (*Delphinium cultorum* Voss) is classed as a typical bumblebee flower, for it is claimed that the members of the genus *Delphinium* are largely, or wholly (*e.g. D. elatum*), dependent upon bumblebees for pollination (*cf.* Müller, 1873; Knuth, 1898, and Lovell, 1918). In the case of *Delphinium consolida,* Darwin (1876) found that plants covered with "a thin net" produced only about half as many seeds as those pollinated by bees.

In order to determine the effect of the exclusion of bumblebees upon the fertility of *Delphinium cultorum* Voss, the following experiments were carried out by the writer. On the 19th of August, 1923, fifty-four terminal racemes of *D. cultorum,* each having about a dozen large buds, were enclosed in paper bags so that bumblebees would be excluded from the flowers. A few days later, Professor Edward M. East, of Harvard University,

[7] *E.g. Andrena wilkella* Kirby, *Halictus forbesi* Robertson, and *Megachile melanophea* Smith; det. Dr. J. Bequaert.

called the writer's attention to the fact that in some cases this method of enclosing flowers keeps plants from setting seed properly. On August 25, five large plants of *D. cultorum,* each having a considerable number of racemes in various stages of development, were therefore completely enclosed in 1/16 inch mesh wire cages, after the number of flowers and buds of each raceme had been recorded on cards. All the enclosed racemes—including those in the paper bags—were harvested on September 29. The results of these two experiments are shown in the following table:

TABLE I

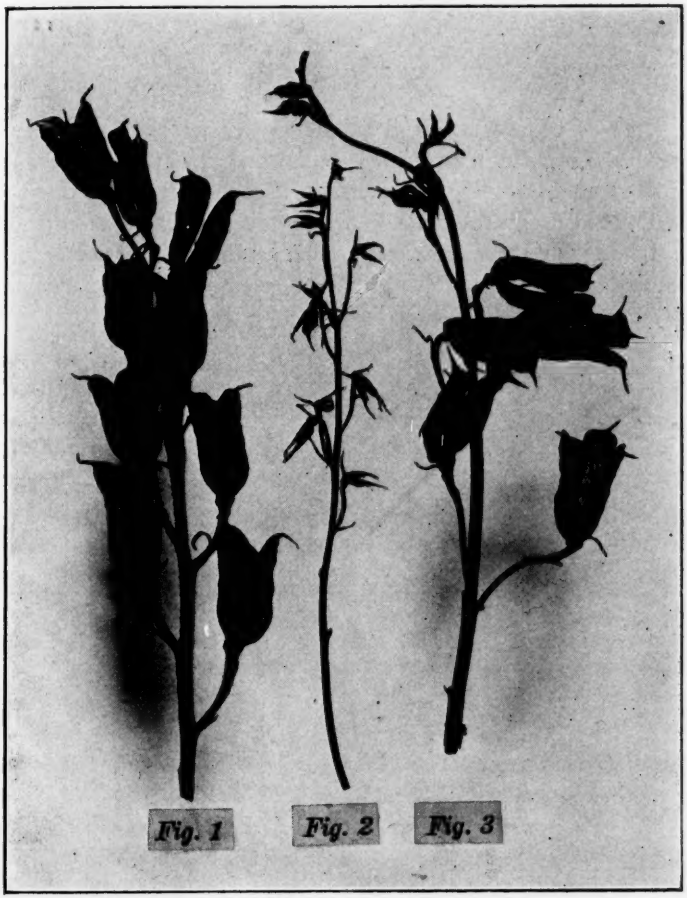
Experiment No.	Number of racemes having buds only	Number of seeds expected[8]	Number of seeds produced
1	54	42,768	17
2	35	27,720	21
Totals	89	70,488	38

The importance of insect pollination in the case of *Delphinium cultorum* Voss is also very strikingly illustrated by the three accompanying figures.

It will be noticed that these results differ considerably from those which Darwin (1876) obtained in his experiments with *Delphinium consolida,* but approach very closely to what one should expect in the case of *Delphinium elatum,* which is regarded by Müller (1873) and Knuth (1898) as being incapable of self-pollination. The few seeds which were obtained in the case of *D. cultorum* might very well be due to accidental pollination by small ants, of which large numbers were observed on the flowers of some of the enclosed plants. A similar explanation may account for Darwin's (1876) results, although it is, of course, possible that *Delphinium consolida,* unlike *D. cultorum* and *D. elatum,* is capable of self-pollination.

In the vicinity of Boston the flowers of *Delphinium cultorum* Voss are pollinated largely, if not exclusively, by

[8] Estimated at the rate of 22 seeds per follicle and 36 follicles per raceme.



The above figures represent parts of three racemes which were used in experiment No. 2. At the time they were enclosed (August 25, 1923), the flowers of these three racemes had reached the following stages of development: Fig. 1. All the flowers had been in bloom for several days; Fig. 2. The flowers began to bloom on August 26; Fig. 3. Only the lower flowers were in bloom.

bumblebees, of which *Bremus* (*Bombus*) *affinis* and *Bremus fervidus* are the most common visitors. The last-named species obtains the nectar of *D. cultorum* in the

normal way, but the workers, as well as the queens, of *Bremus affinis* invariably secure it by perforating the spur, and hence probably play no part in the pollination of the plant.

Later in the summer, bumblebees, other than the males and workers of *Bremus fervidus,* are rarely seen on *D. cultorum.* We have here, therefore, at least during a certain part of the year, a similar state of affairs as in the case of *Delphinium elatum* which, in certain parts of Europe, is said to be entirely dependent upon *Bremus hortorum* for pollination (*cf.* Müller, 1873; and Knuth, 1898).[9]

IV. Bumblebees and the Tomato Crop

Not long ago the following account appeared in one of the Boston papers under the heading, "Bumblebees wanted, or the tomato crop will fail":

> Bumblebees are wanted to save the tomato crop of the Northwest truck growers. There is a shortage of these black, fuzzy fellows this summer, which according to botanists are necessary in the work of pollination of the tomato blossom.
>
> Truck growers and gardeners report to the State Department of Agriculture that the fruit of the tomato will not set because of some sort of blight affecting the blossom, which falls soon after opening.
>
> The theory advanced by the ranchers is not correct. The blossom falls because the bumblebee, a specialist in the system of correctly scattering pollen, has for some unknown reason disappeared this season.
>
> It is reported from several localities in the Northwest that field mice prey on bumblebees and devour the eggs while in the comb.

The assertions in the preceding quotation are contradicted by the results of Fink (1896), who found that the flowers of the tomato (*Lycopersicum esculentum* Miller) pollinate themselves if "confined under a screen." This is corroborated by the results of some experiments[10] conducted for another purpose during the summers of 1923 and 1924 by Professor C. T. Brues, of Harvard Univer-

[9] In the eastern Pyrenees, Bonnier (1879) found this plant also visited by other bees.

[10] These experiments are being conducted with the aid of a grant from the Elizabeth Thompson Fund.

sity, who kindly permitted the present writer to make use of these data. In the course of Professor Brues's experiments, various Solanaceous plants, including two varieties of tomato, were enclosed in 1/16 inch mesh wire cages, and yet these tomatoes, as well as several plants of the genus *Solanum,* produced a large crop of fruit.[11]

V. Summary and Conclusions

(1) Darwin's maxim, "the more bumblebees, the more fruitful red clover," probably applies to most, if not all, parts of the world.

(2) The hardy garden larkspur (*Delphinium cultorum* Voss) is dependent upon insects for pollination and—in the vicinity of Boston—is pollinated largely, if not exclusively, by bumblebees.

(3) The tomato crop does not depend upon pollination by bumblebees, but it is probable that the yield is slightly increased if the plants are cross-pollinated (*cf.* Bouquet, 1915).

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[11] Since writing the above, Professor Brues has called my attention to some experiments by Bouquet (1915), who found that the yield of the tomato is slightly increased by cross-pollination.

[12] In order to keep the bibliography within reasonable bounds, it was thought best to omit some of the works cited in the text. They may be found by consulting the extensive bibliography in Knuth's (1898–1905) "Blütenbiologie."

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TRIASO-JURASSIC PLANT EVOLUTION AND CLIMATE[1]

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THE geologic lapse of time considered with respect to plant change and climate lies between the stressed close of the Permian and the equable environments of the Lower Cretaceous forests. Measured in years, this period is about the equivalent of Cretaceous-Tertiary time, that is, from eighty to one hundred million years or longer. It is the time of change from a merely ancient rather than archaic plant world into that of to-day. For post-Jurassic time is only an era in which modern types fix themselves in view, and then by much further evolution amongst visible types enter a great course of dispersal and also displacement of all the more conspicuous coniferous, ginkgoid and especially cycadeoid vegetation which it is here wished to scan for clues to climate.

Taking all time from the earliest plants down, in some arbitrary sense, the age of algae is followed by the archaic plants of pre-Devonian and Devonian time; the ancient canopies of the Carboniferous and Permian end in the transition types leading into present-day vegetation during Triaso-Jurassic time; and finally the rise and dispersal of the purely modern types take place in Cretaceous-Tertiary time. That is, these terms or ideas stand juxtaposed to the great geologic and climatic eras. The era of primordial algal plants is the Algophytic, extending up into Cambrian time; of the early land plants leading into the well-marked forests of the Devonian, the Eophytic; of the upper Devonian types the Archeophytic; of the better known floras of the Mississippian, Pennsyl-

[1] Presented at a symposium on "Ancient climates" held in connection with the meeting of Section E, American Association for the Advancement of Science, Washington, D. C., January 2, 1925.

vanian and Permian, the Paleophytic; of Triassic-Jurassic time the Mesophytic; of Cretaceous and later time the Cenophytic.

Naturally and fortunately there are two apposite viewpoints from which these larger eras may be examined for the facts bearing on fossil climates; although even so, study must for long years to come largely end in the dialectics of inference. First, there is dominance as represented in the successive collections of fossil plants or the primary facts of geographic distribution; and, secondly, there is the much more recondite field of progressive evolution to scan—the actual advance in leaf, stem and floral types. But whichever way the investigator may turn he soon faces unscalable heights as yet lost in the clouds of doubt, and even of fancy. And yet the data of age and area, using these terms in the simple dictionary sense, are being extended; while the greater facts of specialization and extinction are being slowly disentangled from the history of the leaf, the stem and the flower as measured in the types that have prevailed. Especially, too, no one now thinks of any other than a continuous process of change in which for every more visible crest of the wave there must be the beginning and the subsidence, with lateral limits leading toward yet other crests. And were the view continued far enough some of those crests would be found small and local, others of great area. Although that which remains hidden in whatever partakes of the nature of pulsations or waves in plant change as in a sense correspondent to the diastrophic view of continental and climatic evolution, reaches immensity.

In confining attention, then, to some given stretch of climatic time it seems as much needed to search for the evidence of persistence of type as for the high lights of change. Paleobotanic science may be fairly claimed to have progressed at least that far. That is, in seeking for the data of fossil climates every angle of approach must be considered, and all that is inferential must be elimi-

nated or at least set in its proper place. Unless the general extent and nature of change are both held in view fossil climate can not be well adjudged. And then, when the evidence from stems, leaves and flowers is somewhat brought into view, perhaps we may better judge whether Triassic-Jurassic climate was as unchanging and profoundly tropical as long thought, or whether this was just a period of ordinary earth-climate.

DICOT STEM STRUCTURES

Fortunately for the normal development of scientific ideas, through which the final divination and proof of scientific truth may be attained, even smaller groups of workers soon reach complementary hypotheses. And these quickly bring into view the more irreconcilable data. Thus all present theories of dicot stem structure rest on very limited evidence—are beset by two utterly opposed views of conifer stems, and likewise two views of magnolia stems, which are worldwide apart. There is no certainty that Araucarians are the most or the least specialized of cone-bearing gymnosperms, no accord as to whether Trochodendron, Drimys and the magnolias are primitive or not. An acceptable theory of dicotyl stem structure is plainly of the future. When agreement is reached it will mean that much more evidence has been assembled, that the manner in which dicotyl stems were evolved is virtually seen.

Nevertheless, I shall not hesitate to say I view the Araucarians to be old as pines, that I believe the resemblance of Trochodendron wood to a hard pine is real, that the resemblance of the pitted wood of Drimys to that of cycadeoids and araucaroids is also real, and not due to any such veiled course of secondary change as a general later fusion of pits into scalariform markings. And I believe, too, that most of the failure amongst botanists to agree in their interpretation of structure origins here is due to persistence of many old lines and a far more intense parallelism than a slender fossil record can possibly reveal.

Accordingly, it is suggested that from the close of the Permian to the opening of Cretaceous time, change in stem structure from the basal gymnosperm complex into the dicotyls occurred in somewhat the following order: (a) shortening of tracheids, (b) frequent segmentation of scalariform into circular pitting, (c) pith reduction to a certain extent with development of medullar and radial storage tissue, (d) merely relative enlargement of short types of circularly pitted tracheids with loss of borders, and actual reduction of adjacent elements to the fiber tracheid, (e) a course of wood ray development, (f) development of extreme branching, the sparse-leafed shoot and later leaf and floral change.

Differentiation of vessels especially has had some attention from Bailey, and appears related to a very general course of tracheidal shortening long under way. Incidentally, the great tracheids of the seed fern Lyginopteris with their multiseriately bordered pits have some of the features of vessels. The pits are indeed radial, but tangent pitting is not unknown in older woods.

The cordaite-conifer-dicotyl view of descent of a few years since left very great structure gaps to fill. But as discovery has gone on this view has given way in the minds of many botanists to other views, such as the gnetalean view, and especially the cycad or the coniferocycadeoid view with the corollary of ancient lineage. And of course these later views mean consideration of more varied types, so that the dicotyl stem, just as in the case of the flower, is less a mystery than earlier. The gap to bridge is not so great, and discovery of the stem structure of the Triassic *Wielandiella* and of the Jurassic *Williamsoniella* is eagerly awaited. These stems must approximate very nearly a type of stem, with at times certain conifer features as well, which was very widely distributed at the break of the Trias, and which was then actually changing toward true dicotyl structure. Towards the close of the Jurassic the greater course was about ended in defined dicot stems. Thus, in the lower

Cretaceous *Aptiana* the small vessels scarcely disturb the regular radial sequence, are little larger than wood fibers and nearly as elongate as normal tracheids. The medullary rays—those leading out from the pith, have squarish cells like cycads, while scalariform wood is a striking feature in not only *Aptiana* but several of the group of Cretaceous forms described by Stopes.

Closing this brief word, it may be confidently asserted that the generalities to which any discussion of dicotyl stem origin must be confined will soon be replaced by evidence. It is only recently that the older lignites have been successfully sectioned, and it is amongst the many Triassic and Jurassic lignites that the abundant dicotyl record yet lies hidden. The conifer stems have seemed so ubiquitous as to suggest a sole and only record. But this is clearly due to their resinous character, which for centuries prevents decay and seems to aid in silicification. And then too so many of the petrified conifer forests are the great and specialized pure stands which from Triassic times down lived on low plains on the edge of the desert where easily engulfed by drifting sands with abundant silica. One of the world's greatest petrified forests for all time, that of the Chinle above the Shinarump in Arizona, has both extent and persistence in time. There the pure stands of araucarians reaching 200 feet high are petrified for hundreds of miles around as if in a full totality, and the entire surroundings, with every geologic feature as noted by Gregory indicate the edge of the desert. And those southwest deserts of a warm temperate climate, as so well marked at the beginning of the upper Trias are again noted at the break of the Jura. But with their great xerophyllous pure stands facing the desert as the Argentine araucarians face the desert and the scrub to-day in behind the Patagonia plateau, they seem to have continued almost unbroken from Shinarump to Como time, leaving behind petrified forest after petrified forest of conifers. And it was during that long stretch of time and desert, in all likelihood with its Euro-

pean counterpart, that the heavy-stemmed cycadeoids were segregated from their numerous kind and reached the stem bulk and feature stamping them as desert plants.

Monocot Stems

The monocots, like the dicots, must have reached a strict modernity of stem and flower by the advent of Cretaceous time; although the direct evidence so far merely indicates that in the upper Cretaceous the monocots were diverse of family and cosmopolitan. While the negative evidence all goes to show that as Newell-Arber seems to have been the first to suggest, dicot foliage is young, the exact contrary seems true of the monocots. The fan type of leaf is of course a later instance of extreme specialization, but the simpler acuminate types are probably as old as monocot history. It is in fact not the leaves or flowers of the monocots but the stems which present to paleobotanists perhaps their very greatest enigma. Until recently no one has even ventured a suggestion of the manner in which the monocot stem may have arisen, unless as a totally discrete structure for all time. But now Noé and Wieland assume that the immersed primary wood of Heterangium suggests a pseudo-monocotyledonous structure, and that the so-called Myeloxylon may include types which have discarded their secondary wood entirely. If vessels could arise in secondary wood, why not in a stem the structural unit of which is the sheathed bundle?

Herbaceous types have been more than once hypothesized as derivatives of woody types; and it would seem little more strange if in the depths of the Paleophyte, where nature seems literally to have experimented with nearly every possible combination of primary and metaxylem, the dicots and monocots were actually in contact. The conifers, too, because of the presence of foliar centripetal wood in the lower Cretaceous Prepinus of Hollick and Jeffrey may once have had perimedullary strands like those of the older Cordaites. It is in brief an amazing fact that although the main lines of seed plants ap-

pear discrete from the oldest Paleozoic on, there has always been an essential unity of structure, a visible parallelism in the forward movement toward the later structural types. At least the monocot stem structure is called to mind in one very important chapter of the history of early stem organization in the seed plants, and from far back in time must stretch across the Trias and Jura, whether the record shows the fact or not. Were the forms more generally tropic than later? Surmise is the better answer.

GROWTH RINGS

The oft-repeated inference or even emphasized statement that absence of growth rings in fossil stems at once indicates higher average cosmic temperature ought to be weeded out of geologic texts and ought no longer be copied by botanists. That growth rings must often yield approximate data for local climatic conditions is evident. But several investigators, amongst whom should be mentioned Antevs, call attention to the fact that the present knowledge of fossil growth rings is very far from quantitative. Rings as yet afford little more than a clue to the more general character of climates back of the Cretaceous. For, although narrow or discontinuous rings occur from the Devonian down, it is only at the close of the Trias that zonation is better marked, and only towards the close of the Jurassic that the modern stem and foliage complexity is reached, with widespread and sharp seasonal response in growth rings. If environment means anything here, these definite end results might rather be thought of as inevitable because seasons have been sharp and climate zonal as long as there have been forests.[2] In

[2] Scott has recently described two stems of the Calomopitys type from the lower Carboniferous of southern Scotland as *Bilignea solida* and *Endoxylon zonatum* of interest here. Both have regular zones of autumn wood, as does the related Mesopitys of the Siberian Permian. New evidence for former desert, cool or cold climates arises continually as stem types increase in number. Also, with more searching examination of older carbonized leaf imprints by "chemical methods," types with cutinized hairs, a protection against dry and cold, are found again and again.

the simpler types of wood before the days of marked tracheidal and especially wood ray tracheid differentiation, growth rings were slow to appear; and even now they result from special and local climates to which they are not always strongly affixed. Tropic species may lack the rings, and yet grow side by side with other types which have well-developed rings. Also rings have been seen in a South African monocot.

In the upper Jurassic coniferous stems of King Karl's land (78° N. L.) east of Spitzbergen, Gothan found rings as well defined as in later Tertiary types. Gothan in this connection remarks that these rings appear in a well-represented group of Abietineans, that no associated araucarians were found, and that in the upper Jurassic of Spitzbergen as pointed out by Nathorst many Abietineans are again present—but there, with nearly total absence of cycadeous plants. There is some evidence for a later southward invasion of Abietineans.

Ring relations in the dicotyls, as Gothan says, much depend on leaf fall. Ring and ringless species grow side by side in the tropics, and weak rings may exemplify local conditions or types of structure in higher latitudes. Schenk found the Cretaceous conifers of the Libyan desert without rings, as would often be the case in other forms with persistent leaves. In general, therefore, the rings in essentially modern stem types do indicate temperature—summer and winter, rather than a wet-dry year. But rings are not very pronounced in the xerophylous south Brazilian Araucaria forest (25° to 30° S.), although more marked in the pure stand Chilo-Argentine *Araucaria imbricata* forest of 38° to 40° S. where a snowy and a dry warm season alternate sharply. Both these imposing forests require altitude, and it is believed that the lower slopes of Lake Chelan cutting back into the Cascades from the East in 48° N. are so exactly a physiographic equivalent of the slopes of Lago Aluminé, soil and all, that merely a free planting of seeds is all that is needed to secure a great growth of the *Araucaria*

imbricata in this yet higher latitude. That means for this forest species an easy sweep poleward of 8° under even present conditions.

Withal, as the dicot stems are so little known, and as the conifers of the Mesozoic were so often leafy and simple of stem, there is nothing in the absence of rings well up within the limits of the present temperate zones which could be taken alone for proof positive of climates much warmer than now—that is, of isothermal variations unaccounted for by the simpler factors of geologic change.

The persistent leaf type has seemed omnipresent in gymnosperms, going back in time, and dicotyl stocks may have been less deciduous in Triassic and Jurassic times than now. But the point had better not be begged. Deciduousness may have been quite as marked in pre-Cretaceous as later forests. Many of the more microphyllous smaller stemmed cycadeoids may have been characteristically deciduous, while there must have been the constant tendency to leaf fall in the dicot lines. And then, too, there were the great circumarctic forests of leaf-shedding ginkgos, and at least up to Liassic times yet other Cordaite relatives of like leaf-fall habit.

It is seen, then, that with relationships amongst the phyla so little known, with an immense preponderance of lowland megaphyllous types in recovered floras, with stem and leaf structure little coordinated, that with the continual uncertainty as to whether the dissociated stems, leaves or fruits are of evergreen or deciduous plants, and with growth rings mainly a feature of modern wood organization, an intense difficulty arises in interpreting the scattered data of fossil rings.

Perhaps the most noteworthy assemblage of later seed plant stems yet brought to light in any country is that from the lower Greensand of the Isle of Wight and nearby, because of the association of both conifers and the oldest found dicotyl stems, described by Marie Stopes. There in N. L. 51° not all the stems have rings, and the more significant is it therefore that Taxoxylon, Podo-

carpoxylon, Pityoxylon, Protopiceoxylon, Cupressinoxylon, all have fairly marked rings. While the dicot *Aptiana,* which may come from Luccomb Chine in association with *Bennettites Gibsonianus* or Cycadeoidea, and which is structurally held near the tea plant by Janssonius and Moll, and near Vaccinium our northern "blueberry" by Bailey (!) also has the rings. Such facts, such structures, taken with the disposition to view the somewhat earlier Oolitic floras as tropic, teach us all to have a care in using growth rings. Or turn to the southern hemisphere; at Portrerillos on the Trans-Andine I found in rocks presumably of Rhaetic age a characteristic Cupressinoxylon with a well-defined transition from spring to fall wood.

If one were permitted the guess of a lifetime as to climates in the region of the present Isle of Wight in lower Greensand time, in so far as an appeal to the evidence from the plants alone is concerned, conditions might be imagined in which there was a certain nearness to arid belts and some wet winter cold. Nor would any one have needed to travel far to find warm blankets of snow. Just a little earlier the sediments of the Wealden deltas carry Cladophlebis, Sagenopteris, Sphenopteris, Ruffordia, the pseudo tree fern Tempskya, Cycadeoidea and Fittonia, Thuyites and Pinites. But these are not certainly more than warm temperate, and it is left to paleozoologists to speculate on possible habits of migrant pterodactyls or dinosaur winter habits. A little earlier still, in the Purbeck, the Cycadeoideas had reached an extreme of specialization and xerophylly.

It must not be supposed for a moment that there were not from ancient times down forest types that could stretch afar into the world's utmost pole of cold as now. With Arctic lands often entirely wooded and low and the controlling barometric areas variable, it is, then, not needed to drag in tropic climate to any great extent, in accounting for early absence of rings. The ring occurrence is still a dark page, yielding inferences, rather than directly or fully readable climatic data.

TRIASSIC-JURASSIC LEAF CHANGE

Any one who wishes to gain some conception of the foliar change that ended in the Angiospermous forests over and above mere opinion, and probably over and above anything as yet readily gained from tabulation, should for himself pore over the plates of the volumes of Heer's "Flora Fossilis Arctica." And it may be suggested that in so doing there should be at hand the Heer "Bibliographie of Malloizel." This is a little book of 176 pages with an introduction by Zeiller and was published in Stockholm separately and without date. It was really completed by A. G. Nathorst after much labor, a fact of which there is no inkling in the title, and still lacks a tabular study of horizons which some competent student of the dicotyls should yet give.

The publication of these volumes was surely one of the most sensational events in paleobotany and left an impression amongst their readers that the polar areas were long the home of much admixed northern and southern types of vegetation, and that many new types had originated there, later to migrate towards the bases of the north continental areas. At present, such ideas though not entirely abandoned no longer appear simple, being merely mentioned. But the scientific world was left to wonder over problems of celestial mechanics; and Heer himself, without absolutely rejecting the theory of descent, had recourse to ideas of brusque modification of type approaching new creations in the organic world not unlike those of the days of Cuvier.

Now amongst those who have attentively examined the volumes of Heer and then turned to the fossil dicotyl forests further south in a very elemental study of foliar evidence bearing on the ancestry and early climatic environment of the angiosperms are Bailey and Sinnott. They have made a compilation of the *palmate-simple, palmate-lobed, pinnate* and *compound,* Cretaceous and Tertiary species, and they reach the following numerical statement:

	Palmate simple	Palmate lobed	Pinnate	Compound
Cretaceous 908 sp.	15%	15%	59%	10%
Tertiary 877 sp.	26%	7%	55%	12%
Woody Plants 7,014 sp.	9%	4%	65%	21%

The palmate types show an early abundance, and even seem to give way to pinnate vegetation. But here follows a more subtle argument from evidence. It is shown that innumerable floral bracts, calyx lobes, petals and cotyledons, are multi- or very conspicuously tri-lacunar in their bundle supply, and yet accompany pinnate and blade types of leaves which are unilacunar, or nearly so. From all this it is possible to suggest that the old angiosperms were palmate often with three separate bundles at the node, later pulled together into the single midrib strand. And Bailey and Sinnott note that among woody plants uni-lacunar forms now seemingly predominate in the tropics, multi-lacunar and especially palmate lobed leaves in temperate and upland regions. Hence the angiosperms appeared in climates more temperate than tropic, found in much of Mesozoic time only in cool to cold uplands. The view is in reality corollary to earlier theories of polar origins, and is held to favor angiosperm descent from a coniferous palmate rather than a cycadean pinnate stock.

The difficulty of tracing the course of early dicotyl leaf change is so enormous that speculations like the foregoing have the highest value. They, and such alone, lead to more accurate consideration of the elements of the main problem. The actual milestones along the way of angiosperm origin are not seen. But there are other possibilities to take into consideration, as for the greater part merely enumerated here. (1) The picture of change from the break of Cretaceous time until now is one of varied climates with much evolution throughout the angiospermous stock. (2) Parallelism or isolated stocks, or polyphylly means those very long lines that stretched

across all Triassic and Jurassic time, ending not in a few but in those many and varied Cretaceous forms. (3) While conifers, cycadeoids and cordaites were distinctively such at the opening of Triassic time, all these groups must have found certain close relationships with near-cordaite, near-conifer and near-cycadeoid types actually leading into angiosperm foliage. Would it be absurd to say that in a larger sense all gave their quota before the break of the Trias, if it is admitted that any one could have done so? (4) While it is true that the various Triassic and Jurassic floras only include, holding in mind a precise chronology, a small fraction of one per cent. of contemporaneous forms for the given point of time, it is unthinkable that paleobotanists have never seen and have never held in hand leaves in the line of descent. There are collections from certain cooler climates like the Argentine Rhaetic, and others from more tropic regions like the Rhaetic (= Keuper) at Lorraine, Virginia; and the suggestion is ever present that many of the leaves recalling Oleandridium, or other ferns, or called Taeniopteris or Nilssonia, or perhaps like the leaves of Williamsoniella or Wielandiella, are merely the foliage of real progenitors of the Cretaceous angiosperms. For it is evident that in the course of dicot evolution leaf change came late or last. (5) The magnolias are a very varied stock far back in the Cretaceous, and probably had pinnate leaves from Triassic times to now. Eames has noted that unique amongst dicotyls so far as observed (but possibly also in Drimys and Trochodendron) the stamens may be added to the Sinnott and Bailey list of floral organs of multilacunar or triadic structure. Three bundles traverse the stamens, and the general floral structure, although without petal or stamen fusion, is elaborate. The magnolia leaves are in agreement decidedly multilacunar.

Only the last of these points as entailing some consideration of bladed types of leaves like Nerium, Sideroxylon, Liriodendropis, Liriodendron and the Magnolias

need serve for present purposes of argument. And in reality the entire question as to the trend of lower Mesozoic leaf change resolves itself into whether a sassafras or a magnolia is the more ancient, with the somewhat primitive ancestry restricted; or whether both are old and had very numerous forerunners at the base of the Trias. In either instance it is easy to picture and to admit that there was some change all through the later Jurassic toward simple blades, and *vice versa* towards palmate leaves. But in the main, and repeating, there is that larger question of palmation and the coniferous ancestry, on the one side, and of blades and a more cycadeous ancestry on the other.

Clearing the way a bit it is observed that unequal rates of change in prevailing groups must always be admitted, and that admittedly the fossil record is so apt to be confined to specialized types and habitats. But starting again from the Flora Fossilis Arctica, it is seen that in the Komé or lower beds of the Greenland series the bladed cycadeoid or Pterophyllum-like form, so very prominent as the old Triassic frond type first to reach world-wide distribution, is still present. And then there is another foliar blade, also old of feature and relationship, the illustrations of which every one might wish to examine for himself. That is the *Nilssonia Johnstruppi* from a little higher up, in the Atané beds which also contain a great Liriodendron bed. Now it is not contended that this latter is not a cycad. But it is a simple type of leaf that has lasted long, and certainly related itself by way of the Ptilophyllums to the extreme or Otozamites types of cycadeoids, culminant over the globe in about Oolitic time. And by the same token there is a relationship to Stangeria of to-day, so far as foliage goes, quite the most primitive of cycads.

Turn, then, to a larger and a greater picture, with the *Nilssonia Johnstruppi* still taken as the turning point, taken as a member, fall where it may, of the Cycadophyta. Perforce any group of plants mainly fossil seems homo-

geneous or natural only with respect to its foliage. But Tumboa, as a member of the Gnetaleans, if only found fossil could not easily be recognized as the kin of Gnetum, more than once suggested as a possible descendant from some of the peculiar leaf types of the Virginia Rhaetic (= Keuper). And so in the Cycadophyta, the stems and fruits remain for the vast numbers of types included, and for the long time range, mainly inferential. The history of the group simply extends back into contact with the generality of older gymnosperms. The Cycadophytes thus include frond types extending from those with pinnules like the existing *Zamia Wallisii* a foot and a half long by a foot in breadth to very narrow linear and diminutive types. Also the double bifurcate condition in *Cycas Micholitzi* suggests by itself and without even the fossil evidence that could be cited, transition toward Ginkgo leaves. Yet venation may be quite fern-like, or again may run toward a fairly typical net, as in the Jurassic *Dictyozamites.* An interesting intermediate form, cosmopolitan in the later Mesozoic, is the genus *Ctenis.* The Cycadophyta thus range from the bipinnate Bowenia to simple blades, like those of the Cordaites. And such may be sparsely inserted on slender stems, even running into whorls after the manner of dicotyl leaves. The Rhaetic Wielandiella splendidly proves the point, and because of the very broad petiolar almost stipulate lamina may even have had a trilacunar bundle supply.

Much more this simple blade of a form like the ubiquitous *Taeniopteris* has been assigned to the remarkable *Williamsoniella,* unfortunately with some doubt as to absolute correctness. But the gap is at best slight, and it is seen that rapid and easy change from cycadeoid towards the simpler dicotyl blades could have early set in. Amongst the many Jurassic so-called Taeniopterids those with a simple marginal vein have the best chance for dicotyl affinity. It is seen that by a simple and regular invasion of the marginal net with alternant weakening of the pinnate veins the Liriodendropsis type of leaf would

arise. And if the stock were somewhat multilacunar there could also be the transition towards sassafras and other palmate types, just as readily as in the reverse direction suggested by Bailey and Sinnott.

An imposing array of transitions can here be suggested. Take the changes exemplified in the magnolias of the Kansas Cretaceous. In *Liriodendron semialatum* the apex is merely obtuse, with a light basal lobing as the name implies. Venation and form may come from the simpler blade of *L. primaevum* Newberry, also present in the Greenland Atané. Then follow *L. Meekii, L. giganteum,* near *L. tulipifera,* which is an old type also. Then, too, there is the *Liriodendron pinnatifidum* with freer lateral lobing, and more retention of bifurcate lateral veins as in some old Ficus types. There is also the pinnatifid *Liriodendron Snowii,* for the removal of which from the magnolias sound reasons fail. What an imposing forest element the magnolias really were down to the Tertiary.

Viewed as a primitive type, or a type surely as old as palmate leaves, the magnolias have both the record and structure in their favor. Taking the young leaf, the venation is for a time even Taeniopteris-like. The mesh develops from the margin inwards, and late. Also, while it could be wished that Bailey and Sinnott had investigated the petiolar supply, it is complex. The bundle alignment is not so very different from that of cycads, while the condition of the stamens above cited is in accord.

If now the point has been scored, if it be admitted that simple leaves are as old as the palmate, that the cycadeoids were an immensely varied frequently deciduous group with real angiosperm contacts, then with other data adduced all these lines are old and in some form traverse all Triassic and Jurassic time. Then, too, it is seen or rather will be admitted that the dicotyl ancestry must be both old and polyphyllic, and it is also easier to grant a somewhat similar status for the ginkgos, and especially the conifers. And if all these things be true a

different picture of Triassic vegetation is reached from that so long in view. There is no longer in sight a great group of only tropic cycads compelling the belief that the pines and Araucarias, the leftover Cordaites, the ginkgos, living with them must all be tropic too. Instead there are the mixed forests of generally polyphyletic origin, all the elements of which undergo more or less forward evolution, and thus acquire a prevailing cast. And these varied and manifold types hold their place stubbornly as they react to ecologic factors, each ever striving towards the farthest reach, the remotest habitat, and all alike subject to the fluctuating geologic climates, the changing shore lines, the flow of warmer, then colder waters into the Arctic areas, and that lure of the desert which so specializes plants that they never get back to the moister climates and are cut off. In a word, all those lines from the Triassic down may be thought of as having the power to send some of their members to the ends of the earth, just as surely as the larch and the pine reach the pole of cold now. And for such weighty reasons few of the Triassic-Jurassic seed plant types should ever be thought of as surely tropic, or surely warm temperate without some weightier reason than inference.

FLORAL CHANGE

A few years ago it was said that the strange thing about the dicotyl advance, as Saporta said, "rapid as universal in its effects," was the flower. And yet when the discussion of natural selection was in its beginnings the Reverend George Henslow had brought out a brilliant book on the "Origin of Floral Structures" in which he ventured the view that, "in a broad sense natural selection seemed obviously true; for Geology had revealed the fact that the world had been peopled over and over again by old forms dying out and new forms coming in; so that although it might account for the extinction of the former, it did not seem capable to account for the origin of the latter." He therefore looked to the environment "as

affording a better clue to the source of variations," and mentions as worthy of serious consideration the idea of inherent tendency to higher organization attributed to Naegeli. And Henslow says he returns to 1795 and the "Monde ambiant" of Geoffroy Saint Hilaire.

That is the view taken here. The stems are not in the way, the leaves are not in the way, and the flower is no longer in the way. As far back as the Pennsylvanian there is an as yet not fully understood adumbration of the modern floral type in *Codonotheca* of the Mazon Creek nodules, and in Rhaetic time *Wielandiella,* also lacking in full details of the disk structure, is nevertheless seen to be nothing but a small flower on the Ranalean plan, pertaining to a very simple type of leafy blade of worldwide distribution all through the Trias and Jura. *Williamsoniella* as a less reduced but also microflorous type of the same order of plants only proves the point. While in the Cretaceous the giant flowers of the heavy-stemmed highly specialized cycadeoids at once prove the presence of very ancient lines and show that an essentially angiospermous floral plan held true in the varied types of Mesozoic cycadophytans.

When the flower of *Cycadeoidea ingens* was found the *Williamsoniella* was unknown, and the *Wielandiella* was yet to receive the later study of Nathorst, so that for a time the idea of later floral structure as a result of extreme reductions may have been overemphasized. But at present any such ideas are very much tempered. It is seen that virtually a flower has always been a flower back to the time that many cycads and pines compacted their sporophylls into cones as distinguished from the kind of sporophyll emplacement called a flower in the modern sense. It is better understood that the "sterilization" of which Bower speaks even antedates the leafy terminal cones of the club mosses which are the remote prototypes of modern cones and flowers. And accordingly it becomes very possible that the flowers of the greater cycadeoids, and alike the great cones of Macrozamia weighing sev-

enty to ninety pounds, or the cones of *Pinus Coulteri,* are examples of a secondary gigantism; although this offers no difficulty. The superb flowers of the Himalayan *Magnolia Campbelli,* a foot across (and Magnolia flowers a foot across grow in our own climates, latitude 41°, accompanying leaves 29 inches long), would be no more remarkable, taken as later giant flowers. The triadic staminal bundles of *Liriodendron* noted above are probably general in the magnolias, and show at once the presence of old structure and the ease of variation in sporophyll development. Eames, who has made extended studies of flower histology, independently reaches the view that the magnolia flowers have the tendency to increased size, and he also emphasizes a belief that the varied structure of dicotyl flowers indicates extreme polyphylly.

Put in few words it is then certain that some kind of floral plan essentially ranalean in many small-flowered forms characterized the enormously varied cycadophytans of lower Mesozoic time. And bearing in mind that the recovered fossil record is usually only a fraction of a per cent. of the actual plant life, it is again emphasized that much of the so-called cycadophytan foliage of Triassic and Jurassic times is that of plants near or within the actual line of dicotyl descent. But obviously the larger and more specialized cycadeoids, particularly the Otozamites, Podozamites, Ptilozamites and most typically Williamsonian types, must here be as rigidly excluded from the view as the cone-bearing cycads, and all the post-Permian cone-bearing gymnosperms. It is the deciduous microphyllous, microflorous branched cycadeoids that alone could stand near to, or share in lines of descent; while the geologic-paleontologic fact of a scant and hidden floral record is without significance. No one dares suggest that proportionate to diversity of species in older, somewhat simpler forest alignments, flower growth was not cosmopolitan from the Permian on. It may be suspected, however, that just as the discoveries of cycadeoids have changed all views of cycadeous foliage, so some

of the older leafy conifer foliage may pertain to flower-bearing forms. The point made here is that cycadeoids flowers, like the leaves, suggest full adaptability to varied climate, though known in so few forms, and so far in no case outside of Cycadeoidea tied up with stem structure.

Assumption that Large Leafed Ferns are Tropic

So far as can be judged from the approximate 6,200 fern species of to-day, most large-leafed ferns with either simple or compound fronds have always lived in either tropic or moist habitats. But in considering Triassic and Jurassic genera there must be a very large margin of doubt as to the climates indicated by the ferns. Ordinary or true fern foliage being so much like that of the seed ferns, it is inferred that as seed ferns finally disappeared in the Jurassic they were replaced in many forests by simpler fern types like those of to-day with incidental specific variation. Furthermore, as proangiospermous forests merged into the Cretaceous angiosperms, yet another world was opened to the ferns—that of the net-veined canopy. The richer stores of humus and more varied shade conditions meant a final variation into the present fern groups.

Accordingly ferns may be looked upon as far more numerous now than at any preceding time, while precisely the period under consideration must cover a very varied course of fern change just as the record seems to indicate. Inasmuch therefore as the ecology and constitution of the lower Mesozoic fern forest is seen to vary along definite lines, it is affirmable that fern foliage affords merely indirect evidence for tropic conditions of much greater extent than now. Thus what the Dipteris-Matonia group was like in the older and differently constituted forests of the past is a problem beset by many difficulties of chronology, variation in land masses and uncertainty as to temperature tolerance that always steps in in the case of the extinct species of even the fern families. At first it however seems very compelling to

learn, as Seward points out, that the Dipteris-Matonia alliance, which includes the singularly handsome lyrate ferns of Rhaetic time extending up to the 60th parallel, is now confined to four genera of the Chinese Malayan region. Especially so when it is added that the hitherto mainly European *Laccopteris münsteri* occurs in the form of fine fertile fronds in the Sonoran upper Trias, and that the curiously characteristic Andriania is found in North America for the first time in the basal Lias of Oaxaca. But it is far easier to cite these examples of change than to tell what they mean in exact climatic terms. For instance, the Schizeaceae, known locally and rarely in New England only from the "curly grass" and "climbing fern," once lived over the northern, but now only persist over the southern world, while the Marattia group, so widely extended as a fossil form, North America excepted, now reaches New Zealand and Cape Horn.

Perhaps I here suggest too much and doubt too superficially. But if I may add a word with myself included I may say that I once stood by a small cool spring within about 200 feet of the very top of a forest-clad, bell-shaped mountain reaching an altitude of some 5,000 feet in southern Oaxaca facing the Pacific. Roundabout there was a little fern garden with many lesser species, clustered round one single noble tree fern of a type I had never noted in the wild before and never had the fortune to find again. It seemed in its surroundings quite the handsomest, stateliest plant I had ever seen. The fine globular trunk had the size of the largest of the cycadeoids, while the fronds of the fertile crown ten feet in length I took to indicate a *Marattia laevis*. Such a fern, but not cycad trunk, and such fronds I would take as certain evidence of tropic monsoon climate though found in fossil beds at the pole. But for many lesser forms polypodium-like, for instance, who shall yet say whether all tropic or not?

RÉSUMÉ

Contradictory as such a dictum might appear, it may be asserted without reservation or doubt that the entire

course of fossil plant investigation has reached a point where not only an extreme parallelism and persistence of the greater plant phyla is indicated, but, going back in time a closer and closer contact of phyla. That is why in building up or picturing the long used simile of the "paleontologic tree" discordant views of the lines of descent are invariably reached. The views were never entirely wrong, and yet they are foredoomed to fall very far short of truth. In particular the dominant angiosperms were thought to be very recent in their main evolutionary phase, and therefore sprung from some very primitive or rather generalized and long persistent stirp of now the pines, again the cordaites, or even later the gnetaleans, always hidden away in some far corner of the earth, predestined never to be known.

But a different point of view is reached. There have always appeared, as the ascendant course of plant evolution proceeded, specialized types, generally of giant form and bulking large in the recovered record. Yet the main groups of seed plants have long been discrete and numerous of kind, and in searching for the ancestral forms unequal rates of evolution in the different organs, a point early stressed by Coulter, must be considered. The more does it become necessary to find those types of the pre-Cretaceous horizons with the possibility of restoration; the more is it necessary, in the case of fossils like the continually dissociated stems, leaves and fruits of all lower Mesozoic time especially, to engage in renewed search in regions promising critical evidence. Here may be mentioned the great Liassic-Oölitic series of Mexico, the remarkable Rhaetic laminated shales of Argentina, and perhaps some of the outlying regions of a strong distributional interest like the Mesozoic areas of New Zealand indicating mixed floras with some of the early dicotyls.

And much more, a viewpoint is reached where the lines of descent investigators seek to disclose have practical value, instead of mere academic or philosophic import. To the chronology must be added a bettered genealogy

else the paleobotanist is too little guarded against error, too often helpless in any attempt to interpret the climatic data afforded by the lesser, and even some floras of more numerous species. It proves of the utmost interest to know whether a leaf type belongs to a group nearly always tropic or to a more diversified group which had the power to adapt itself to varied climates. It will not do in these climatic discussions to assume that the given type is a gingko, when it may be a pine or a cordiate, a cycad when it is a cycadeoid, or cycadeoid when it may be a full fledged angiosperm. Much of the ancient vegetation, long thought of as only tropic because found in the coal beds or on the limestone-dolomite latitudes and without growth rings, must from ancient days down have had close relationship to or have included types that could grow in the colder climates.

It is perhaps a question on the side as to whether the later dinosaurs could live only in climates like that of the Vero man, Elephas and the last of the saber-tooths or further north in the temperature of the Okeefinokee or of the Dismal swamp region. But perhaps the real point which begins to appear on the paleontologic horizon is not that the climates in which the Dinosaurs lived were worldwide, but that they were of the "asylum" type.

Plant data have been late of accumulation because the localities of critical importance are far flung, the materials often difficult to investigate, and workers few as compared with the vertebrate and invertebrate students with their large resources. Nevertheless a turning point is reached where the plant data are viewed from the possibility of fixed poles and of uniformitarianism. It is fairly evident that the plant alignments of the past may mean but the smallest variations in the general climatic mean for the globe. Probably the largest single contribution that the paleobotany of the future is ever destined to make is a fair determination of the limits or else the disproof of the Knowlton thesis of earlier earth climates dependent on a diminishing factor of interior heat.

SHORTER ARTICLES AND DISCUSSION

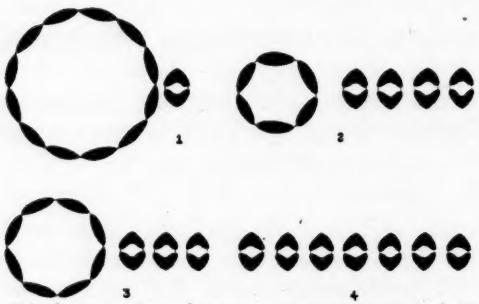
CHROMOSOME BEHAVIOR DURING MEIOSIS IN THE POLLEN MOTHER CELLS OF CERTAIN OENOTHERAS

In recent papers, I have called attention to the peculiar ways in which the chromosomes of Oenothera species behave during meiosis in the pollen mother cells. Six species have been reported on, two of them in detail, and the others in a preliminary way.[1,2,3] Attention has been called particularly to the arrangement of chromosomes in the stage just before the first maturation metaphase, and to the peculiar way in which equal distribution of the chromosomes to the poles is effected. In all the species so far described, the univalent chromosomes during "diakinesis" are found to be so grouped that one or two circles are formed, made up of univalent chromosomes placed end to end. Individual chromosome pairs, such as are characteristic of the cells of most organisms at this period, are present in varying degree, or entirely absent, depending upon the species. Throughout the first maturation metaphase, the circles remain intact, and present a peculiar zigzag appearance, due to the fact that adjacent chromosomes become attached to spindle fibers leading to opposite poles. An equal distribution of chromosomes to the two poles is normally the result of this process.

I have now to report briefly upon five additional species, *Oe. Lamarckiana, Oe. rubrinervis, Oe. rubricalyx, Oe. blandina,* and *Oe. deserens.*

Oe. Lamarckiana: The material upon which these studies were based was obtained from the garden of Professor Geo. H. Shull, at Princeton, New Jersey. Collections were made during the summers of 1923 and 1924. Three strains were examined, all three being derived from plants collected in 1905 by Professor de Vries, at Hilversum, Holland, at the same place at which he obtained his original material. A number of individuals were

[1] Cleland, R. E., *Amer. Jour. Bot.* 9: 391–413. 1922.
[2] Cleland, R. E., *Bot. Gaz.* LXXVII: 149–170. 1924.
[3] Cleland, R. E., AMER. NAT. LVII: 562–566. 1923.



FIGS. 1–4. Diagrams to show chromosome arrangements in late heterotypic prophase. 1, *Oe. Lamarckiana;* 2, *Oe. rubrinervis;* 3, *Oe. rubricalyx;* 4, *Oe. blandina* and *Oe. deserens.*

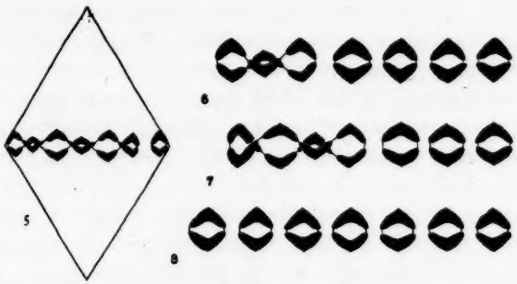
examined. The three strains have proved to be alike cytologically, as have also the collections from both years.

In "diakinesis," the 14 chromosomes in this species are found to be so grouped that they form a large closed circle made up of 12 univalent chromosomes strung end to end; plus one pair, which at first is linked to the circle, subsequently breaking away from it (Fig. 1). In the heterotypic metaphase, the circle remains intact, and in a large majority of cases the chromosomes that lie adjacent become attached to fibers passing to opposite poles, so that the peculiar zigzag arrangement already described for other species is found here also (Fig. 5). It is interesting to note that this arrangement is the same as that found in *Oe. franciscana sulfurea.*

These results are at variance with those recently reported by Boedyn.[4] According to this author, early diakinesis stages in *Oe. Lamarckiana* show seven double chromosomes, often entirely separate from one another. The single chromosomes making these up may split apart partially during this stage, sometimes to the extent of forming rings, but at all events, remain quite closely paired. With the exception of the one pair always present, however, I have found no pairing of chromosomes in diakinesis. Furthermore, every complete nucleus that I have examined, in which all 14 chromosomes could be clearly seen, has revealed without question the circle of 12.

Oe. rubrinervis: Material of this species was obtained in Professor Shull's garden in 1923. The strain is a selfed line of

[4] *Zeitschr. f. Zellen u. Gewebelehre* I²: 265–277. 1924.



FIGS. 5–8. Diagrams to show chromosome arrangements in heterotypic metaphase, side view. Outline of the spindle omitted, except in Fig. 5. 5, *Oe. Lamarckiana;* 6, *Oe. rubrinervis;* 7, *Oe rubricalyx;* 8, *Oe blandina* and *Oe. deserens.*

plants, descended from seed received from Professor de Vries in 1905. My collections were rather small, being confined to one plant, and unfortunately I have been unable this year to obtain material with which to confirm the results gleaned from last year's collection. Hence the results here indicated are to be considered as tentative only. Diakinesis stages in the plant examined showed a closed circle of six chromosomes, with four pairs in the form of rings (Fig. 2). My material was not sufficiently extensive, however, to enable me to determine how these rings were linked together. Beginning with the heterotypic metaphase, the cells of this plant, in many cases, showed a most striking succession of irregularities, the result of which could scarcely be other than the formation of functionless pollen grains; although in some cases, the regular arrangement shown in Fig. 6 was presented. While I am reasonably confident that the suggested arrangement of chromosomes in diakinesis will turn out to be the normal one for the species, I am not at all convinced that these subsequent irregularities are normal, and must wait until I can obtain further material before determining definitely the truth of the matter.

Oe. rubricalyx: Material was obtained from Professor Shull's garden during the summers of 1923 and 1924, from a strain which has bred true for the *rubricalyx* character, and which originated from seed received in 1914 from Sutton and Company —this seed in turn having been derived, by selfing, from a strain sold to that firm in 1912 by Professor R. R. Gates. Collections were made in 1923 from but one plant, but in 1924 they were

taken from a number of individuals. Results from the various collections are in perfect agreement. During diakinesis, the chromosomes are arranged into a closed circle of eight univalents, plus three pairs (Fig. 3). The pairs are in the form of rings. During the heterotypic metaphase, the circle remains unbroken, and the zigzag appearance previously described is found here also (Fig. 7).

Oe. blandina and *Oe. deserens:* Extensive collections of these species were made from plants grown at the University of Pennsylvania, from seed sent me in 1923 by Professor de Vries. Quite a number of individuals of both species were studied cytologically. Both show in diakinesis a perfect pairing of chromosomes. No large circles are present (Fig. 4). The pairs are ordinarily in the form of rings. In the heterotypic metaphase, the pairs line up in a perfectly regular manner, and the chromosomes are separated as they are in most plants and animals (Fig. 8). These two species are the first that I have examined that show 100 per cent. pairing. The significance of this fact I shall try to point out in a subsequent paper.

The species that I have mentioned in this paper are closely related to one another. *Oe. rubrinervis* is a "half mutant" from *Oe. Lamarckiana. Oe. deserens* makes up approximately one third of every culture from selfed *rubrinervis. Oe. rubricalyx* arose from a culture of *rubrinervis* in 1907.[5] *Oe. blandina* arose in 1908 in the 4th generation of the cross *lata* × *semilata,* and according to de Vries[6] represents the union of two *velutina* gametes, derived from *Oe. Lamarckiana,* but lacking lethals, as well as being slightly modified in other ways.

The species or varieties that I have studied cytologically now number eleven. Two show perfect pairing (*Oe. blandina* and *Oe. deserens*), one has a circle of four chromosomes, and five pairs (*Oe. franciscana*) and one has a circle of five, and five pairs, with frequent modifications (*Oe. oblonga*); one has a circle of six, and four pairs (*Oe. rubrinervis*), one a circle of eight, and three pairs (*Oe. rubricalyx*), two a circle of 12 and one pair (*Oe. Lamarckiana,* and *Oe. franciscana sulfurea*), and three of them have no pairing at all (*Oe. biennis, Oe. biennis sulfurea* and *Oe. muricata*). Although we have here a very

[5] Gates, R. R. "The Mutation Factor in Evolution," London, 1915, p. 103.

[6] *Biol. Zentralbl.* 43[2]: 213–224. 1923.

striking variety in chromosome arrangement in diakinesis, the uniformity with which an arrangement is maintained in a given species is just as striking; as is the uniform presence of the zigzag arrangement of chromosomes during heterotypic metaphase in all species possessing large closed circles.

A discussion of the possible relation of these facts to the problems of linkage, crossing-over and the origin of "mutants" will appear shortly, and detailed accounts of the various species will be forthcoming as rapidly as time permits.

It is a pleasure to acknowledge my indebtedness in connection with these studies to Professor Geo. H. Shull, Professor Hugo de Vries and to Professor Rodney H. True.

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A NEW LEG MUTANT IN DROSOPHILA MELANOGASTER

DURING the experiment of the author with Eyeless, a number of flies appeared with shortened and thickened tarsal joints. It seems best to give this character a name in order to make its discussion easier. So large a number of mutants have been described in *Drosophila melanogaster* that it is rather difficult to choose a name that will not conflict with one previously used. In this paper we shall refer to this new mutant as Thickened.

A pure stock of Thickened was prepared. A cross with Star Dichaete showed that the gene for Thickened was recessive and located in the second chromosome. One culture was arranged to give crossing-over from Star, and this showed a cross-over of 35 per cent., indicating that the gene was near Black. A Black-thickened-vestigial stock was therefore prepared.

In the preparation of this stock Black-purple-vestigial was used. Only cultures prepared from double cross-overs, that is, those that had split out purple, gave Black-thickened-vestigial flies. This of course indicated that Thickened is located between Black and Vestigial and near Purple.

Black-thickened-vestigial flies were crossed to wild and the F_1 females back-crossed to Black-thickened-vestigial. The results are summarized in the table.

TABLE SHOWING THE CROSSING-OVER BETWEEN BLACK, THICKENED AND VESTIGIAL

		Black and Thickened		Black and Vestigial		Thickened and Vestigial	
Culture No.	No. of individ.	No. of cross-overs	Per cent.	No. of cross-overs	Per cent.	No. of cross-overs	Per cent.
1	196	13	6.6	35	17.8	26	13.3
2	210	12	5.7	13	6.2	1	0.5
3	247	15	6.1	31	12.6	16	6.5
4	181	12	6.6	33	18.2	25	13.7

It is evident that a cross-over modifier has been involved in culture 2 and probably in culture 3. Sturtevant (1919) has shown that such modifiers exist. This one, however, has not affected the crossing-over between Black and Thickened, so the average of the four cultures should give something close to the correct value. This is 6.2 per cent. The average cross-over percentage between Thickened and Vestigial in cultures 1 and 4 is 13.5. Bridges and Morgan (1919) give 6.2 as the cross-over percentage between Black and Purple, 11.8 per cent. between Purple and Vestigial, and 17.8 per cent. between Black and Vestigial. Thickened is therefore very near to Purple.

In viability Thickened seems to be fair, being fully equal, if not better than Vestigial in this respect.

Stocks of this mutant can be obtained from Dr. W. M. Barrows, Department of Zoology, Ohio State University.

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